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A QUANTITATIVE STUDY OF THE INFLUENCE OF OXYGEN AND TEMPERATURE ON THE EMBRYONIC DEVELOPMENT OF THE EGGS OF THE PIG ASCARID (*ASCARIS SUUM* GOEZE) *

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Hallez (1885) noted that the deprivation of oxygen prevents the formation of the embryo of *Ascaris equorum*. Zavadovsky (1916) has shown that in water swarming with aerobic bacteria the eggs of this same parasite are arrested in their development in the two or four cell stage. Martin (1913) has made analogous observations on the eggs of *Ascaris vitulorum* maintained at 33°C. in water which had been boiled for a long time and introduced into tubes covered with oil. The eggs of this parasite had undergone no division at the end of three and one half months. As parasites become more closely confined, the necessity of free oxygen is apparently decreased. Viviparous nematodes in fact live in cavities or tissues where oxygen is relatively rare, such as intestine and muscles (Trichinella); nodules of the esophagus or stomach (Spiroptera of dogs); muscles and nodular tumors (Onchocerca of man). The embryos develop in the uterus of the female worm and often hatch there. It is possible that they obtain part of the necessary oxygen by breaking down different substances found in their own bodies. The respiration of these worms is poorly understood. Whatever be the mechanism of respiration it is quite evident the necessity of free oxygen decreases more and more and comes to a minimum in nematodes of the most complete adaptation to parasitic life.

Manifestly the difficulties in the way of studying experimentally the development and oxygen requirements of the embryonic stages of viviparous nematodes are quite insurmountable. A rather extensive literature, however, is to be found on the helminth eggs that undergo their development in the exterior, including a small amount of work on their temperature and oxygen requirements. The eggs of different species of Ascaris have been the favorite material for such studies on account of their abundance and the ease of procuring them. As stated

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above it has been definitely demonstrated that Ascaris eggs cannot develop in media entirely devoid of oxygen. The minimum oxygen tension necessary for development has never been determined for eggs of any species of Ascaris. The nearest approach to this is the statement by numerous authors (Zavadovsky, 1916; Wigdor, 1918, and others) that Ascaris eggs will not develop in a medium teeming with bacteria, which they consider to be entirely deprived of oxygen. The actual amount of oxygen present in these cultures was not determined, but from experiments done by the writer it appears that practically no oxygen could have been present. That it might have been the toxic products of bacteria growth and not the absence of oxygen that prevented the development of the eggs in these cases has been refuted by a series of ingenious experiments by Zavadovsky (1916). He found that Ascaris eggs will develop in aerated putrified liquid from which the bacteria were removed. Just what effect different oxygen tensions have on the development of Ascaris eggs is not known. Any experiments on this subject must eliminate at once the factor of oxygen consumption by the bacteria and organic materials that usually surround the eggs in culture.

The effect of various temperatures on development have been studied by Martin (1913) and more extensively by Fauré-Fremiet (1913). Their researches show that there are rather definite temperatures at which embryonic development takes place most rapidly. They also noted temperature limits above and below which Ascaris eggs develop more slowly or not at all. The different species of Ascaris vary considerably in this respect. Thus *A. suum* and *A. lumbricoides* will develop only to the 4-8 cell stage at 37°C., while *A. megalocephala* and *Toxocara canis* develop to infectivity at this temperature. Careful studies on the effect of different temperature at various oxygen tensions on the different stages of embryonic development of Ascaris eggs will add considerably to the understanding of their embryonic physiology.

The object of the studies detailed in this paper was to attack quantitatively certain phases of this general problem of oxygen and temperature. As a basis of comparison it was necessary to ascertain the rate of development of eggs at 21°C. and 30°C. in water saturated with oxygen. The next question to be answered was the rate of oxygen consumption and whether or not eggs incubated at 21°C. and 30°C. consume oxygen at a rate proportionate to their development. From these experiments the total amount of oxygen consumed per egg in its complete development was also calculated. Taking the rate of development of eggs in oxygen saturated water as normal, the effects of low and high oxygen tensions on embryonic development was then studied.

METHODS

In studying quantitatively the oxygen requirements of the eggs of *A. suum* it was necessary first to eliminate other oxygen consuming

materials, such as bacteria and organic matter which usually surround the eggs. To accomplish this the eggs were obtained from the first two inches of the uteri of adult female worms and a solution of 1 part formalin to 1000 parts tap water was used as a culture medium. The culturing was done in 250 cc. Ehrlemeyer flasks which, after the Ascaris eggs had been introduced, were sealed by pouring in melted vaseline until a plug one inch long was formed. That this method prevented oxygen exchange with the exterior is evidenced by the fact that flasks, the contents of which were made oxygen free by boiling, when sampled three months later were still devoid of oxygen. In the experiments in which the oxygen tension desired was less than that of normally saturated water, the excess oxygen was driven off by heating over a period of time, until an analyzed sample showed the desired amount of oxygen per liter of water to be present. The flasks were then sealed and when the water had cooled the eggs were injected in a pipette through a small hole made in the vaseline plug, which was immediately sealed. Throughout the experiments oxygen is given as cc. per liter as this is the standard of measurement. It must be remembered that the culture flasks contained only 250 cc. of water, hence when the water of a flask is referred to as containing 5 cc. of oxygen per liter, the actual amount in the flask is one quarter of this or 1.25 cc. The amounts of oxygen per flask in spite of all precautions varied slightly as was shown by analysis for oxygen of several flasks at the beginning of experiments. Likewise in a series of flasks, the contents of which had been boiled to remove part of the oxygen, a certain amount of variation in oxygen content of flasks, all treated the same way, occurred, and must be taken into account in the quantitative work. This variation was never more than 10% of the total oxygen present and is not sufficient to alter the general trend of the experiments. In all the studies a known number of eggs were introduced into each flask. The method used in counting the eggs was as follows. The eggs from the uteri of a number of worms were put in a small amount of water. This was vigorously shaken and then three 0.075 cc. drops of the suspension were egg-counted. In this way the number of eggs present per cc. could be estimated and any number desired could be introduced into the flasks by varying the number of cc. of egg suspension used.

In the early experiments often as much as three or four inches of the worm's uteri was used to obtain eggs. It was found, however, that much more uniform egg development was met with when only the first inch and a half or two inches of uterus was used. In a number of the early experiments the use of more than two inches of uteri led to the introduction of a certain percentage of unfertilized eggs into the flasks. As these do not develop and were shown by an experiment not to consume oxygen they were disregarded in calculations on oxygen consump-

tion. Further, as the eggs of unfertilized females should not be used as they of course will not develop, a smear of eggs from the vagina of the worm was made to determine whether or not the eggs were fertilized. To follow the whole course of the development of the eggs it was necessary in each experiment to use a number of flasks so that one could be opened every day or two to determine the oxygen content of the water and the stage of development of the eggs. The oxygen content of the water was determined by the Winkler method as outlined in Standard Methods of Water Analysis (1923). In general the course of an experiment was as follows. Flasks were filled with tap water and sufficient formalin to make a 1-1,000 solution, total 250 cc. A known number of eggs, all in the undeveloped condition, were put into each flask and the flask sealed with vaseline. Then the contents of several flasks were analysed to find out the oxygen present at the beginning of the experiment. Controls containing only the formalin solution were also made to check any variation in oxygen content of the flask due to other factors than the developing eggs. The flasks were then incubated at constant temperatures, 21, 23, and 30° C. (temperature variations were $\pm$ 1°C.). At intervals flasks were opened for examination of the rate of embryonic development and the oxygen present. During the incubation of the eggs the flasks were thoroughly shaken every day in order to mix their contents and give the whole a uniform oxygen tension. This prevented the eggs from surrounding themselves with a region devoid of oxygen.

In order to put the experiments on a quantitative basis a method is needed of adequately expressing the rate of development. The one most frequently used has been the time it takes for the motile embryo stage to be attained. In this study, however, it was wished to study every stage of development to ascertain any peculiarities in development of any particular stage. If the eggs developed through all the stages en masse, no difficulty would have been experienced, but in some cases 25% of the eggs outstripped all the others by a full day in development. To take this into account the following method of developmental classification of the eggs was followed: One cell; early morula, 2-16 cells; late morula, 16 cell to complete morula; tadpole, incurved morula to early vermiform stage; motile embryo from early vermiform stage to completely formed embryo. It was found that in general it took about an equal length of time to pass through any one of these arbitrary stages. Hence to each stage of development could be ascribed an index number which indicates the relative amount of time necessary to attain that stage of development. The index numbers are as follows: one cell, 0; early morula, 1; late morula, 2; tadpole, 3; motile embryo, 4. By this manner of scoring all the stages in development share in the final figure for the rate of development. To obtain the stage of development of an experimental culture

several hundred eggs were examined. The percent in each stage is then multiplied by the index number of that stage and all the index values added, the sum giving the total developmental picture. Thus when all the eggs contain motile embryos the index value will be 400. The example given below shows the computation of an "index" value for a flask of partially developed eggs.

	One Cell	Early Morula	Late Morula	Tadpole	Motile Embryo
Per cent of eggs.....	1.0	7.0	50.0	41.0	1.0
Index no.	0	1	2	3	4
Index value	0	7	100	123	4

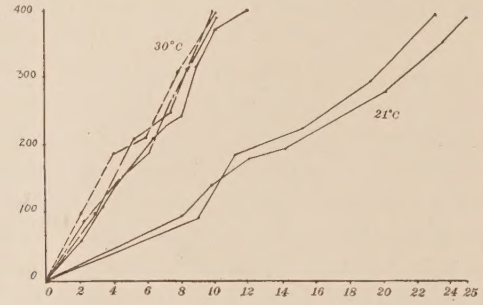
Total index value of development = 0 + 7 + 100 + 123 + 4 = 234.

This method of scoring by taking into account both laggard and precocious development really gives an average picture by including all stages of development and is much more accurate than the old method of merely stating, "many eggs in late morula stage, a few still in the early morula stage," as has heretofore been the method in earlier studies upon the rate of embryonic development of helminth eggs. Further, the index numbers although arbitrary, can be plotted graphically and since every culture is treated in exactly the same way, comparisons on rate of development under different sets of conditions are perfectly valid.

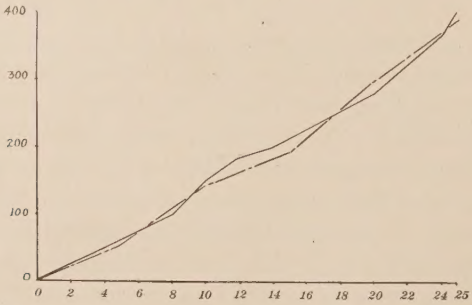
NORMAL DEVELOPMENT OF ASCARIS EGGS AT 21°C. AND 30°C. IN A MEDIUM SATURATED WITH OXYGEN

As the majority of the experiments were to be done either at 21°C. or 30°C. it became necessary to determine first the normal rate of development of Ascaris eggs at these temperatures when incubated in an oxygen saturated medium. This rate then can be used as a baseline for comparison with the experiments in which the conditions are modified. These experiments also determine whether or not the development of each embryonic stage is hastened equally by incubation at 30°C. as compared with that at 21°C. With each experiment on the rate of development of the eggs under sub- or hyper-normal oxygen tensions a control, with normal oxygen saturation was run. Water saturated with oxygen from the atmosphere at 21°C. and 30°C. contains 6.4 cc. and 5.2 cc. of oxygen per liter, respectively. The control series was sampled every time the experimental cultures were examined and they were used as standards of comparison. The developmental curves for a number of these oxygen saturated cultures at 21°C. and 30°C. are shown on graph 1. Each curve represents the development of a single culture and when compared with each other indicate that, given a constant temperature and oxygen supply, embryonic development is quite uniform. There are two sources of error which prevent the different curves from matching even more closely. First, in some of the experiments the eggs were

obtained from further up the worm's uteri than in others and hence some eggs were slightly younger, producing a retarded development. Second, the days on which the cultures were examined were not always equally spaced. It will be seen that some differences will result depending on whether the eggs were examined the day before or after the majority had passed from one arbitrary stage to the next. Despite these two facts and the additional one that classification of the embryonic stages is not absolutely definite it will be seen at a glance at graph 1 that development was quite uniform in the different cultures. In the cultures



Graph 1.—Development of eggs in media oxygen saturated at all times. Graph to left at 30 C., right at 21 C. Ordinates show index value; abscissae, days of development.



Graph 2.—Development of eggs, solid line at 21 C. and broken line at 30 C. plotted on basis of 25 days to become embryonated. Time interval of 30 C. culture increased by 2½ times. Ordinate shows index value; abscissa, days of development.

at 30°C. it took on an average between 10 and 11 days for all the eggs to become embryonated while in those at 21°C. it took from 23 to 26 days. If it is assumed to begin with that lowering the temperature of a culture from 30°C. to 21°C. will result in lengthening the time of development for each separate stage in the same ratio as the increase in total time, then by plotting the curve of development of the eggs at 30°C. on an abscissa increased 2½ times a curve should result which would

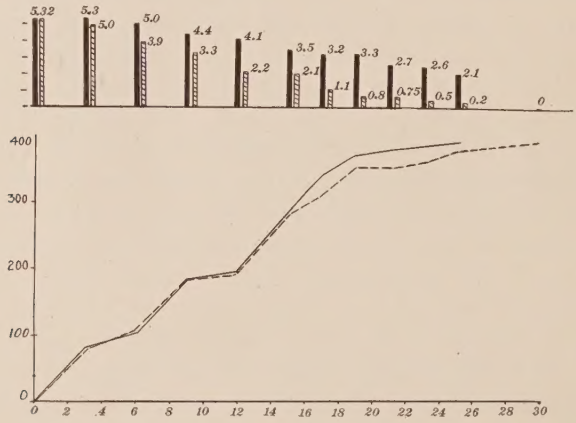
coincide with that of the embryonic development at 21°C. If the two curves did not coincide at any particular points it would indicate that that stage of development was effected differently from the rest by the change in temperature. A glance at graph 2 which shows the development of a culture at 21°C. and one at 30°C. plotted on the same time basis indicates that all stages of embryonic development are equally effected (retarded in development) by the change of 9°C., for the curves coincide with great regularity. The complete embryonation of Ascaris eggs at 21°C. and 30°C. in 23 to 26 days and 10 to 11 days respectively agrees in general with the results of other workers (Ransom and Foster, 1920; Wharton, 1915).

As a general rule it has been found that when a chemical reaction takes place in a single phase, a rise of 10°C. in temperature doubles or trebles its speed. In the development of Ascaris eggs at 21°C. and 30°C. it is found, as noted above, that for an increase of 9°C. the development is speeded up about 2½ times. For each degree rise in temperature then, the chemical activity is increased by 0.277. This coefficient (0.277 per degree C.) fits very well with the above rule for chemical reaction speeds in a single phase. It is well known, however, that Ascaris eggs develop with exceeding slowness or not at all at 11°C. whereas according to the above rule one would expect them to develop in about 60 days. That the development of the eggs at this low temperature does not follow the rule for chemical activity would seem to be due to the nature of their protoplasm. Fauré-Fremiet (1913:647) found that for each 5°C. decrease in temperature, from 35°C. to 8°C., the protoplasm of *Ascaris megalocephala* practically doubled its viscosity, as judged by the length of time required to centrifuge the mitochondria down to the bottom of the egg cell. Hence at the lower temperature the protoplasmic viscosity is very great. According to Fauré-Fremiet's results obtained from centrifuging of these eggs at 2,500 revolutions per minute the time necessary to force the mitochondria to the lower side of the egg cell was four minutes at 35°C., eight minutes at 30°C., 20 minutes at 23°C., 45 minutes at 18°C., and 225 minutes at 8°C. From these data it will be seen that while chemical activity is doubled or trebled with every 10°C. increase in temperature, the viscosity more than doubles with every 5°C. decrease. Thus, the exaggerated viscosity at the lower temperatures no doubt inhibits chemical reaction and hence development as well. It is also well known that the eggs of *A. lumbricoides* will not develop past the 4 or 8 cell stage at 37°C. and degenerate when maintained at this temperature for a period of time, while the eggs of *A. megalocephala* will develop to infectivity at this temperature. This difference can only be explained on the basis of specific differences of their protoplasm.

The foregoing experiments show that at a given temperature in a medium saturated with oxygen, Ascaris eggs develop at a uniform rate. In raising the temperature of the cultures from 21°C. to 30°C. each embryonic stage is hastened an equal amount (2½ times) in its development. Also the increase in the rate of development of the eggs between these two temperatures is roughly similar to that met with in certain chemical reactions, although this relation does not hold for higher and lower temperatures.

OXYGEN CONSUMPTION DURING DEVELOPMENT AT 21, 23, AND 30°C.

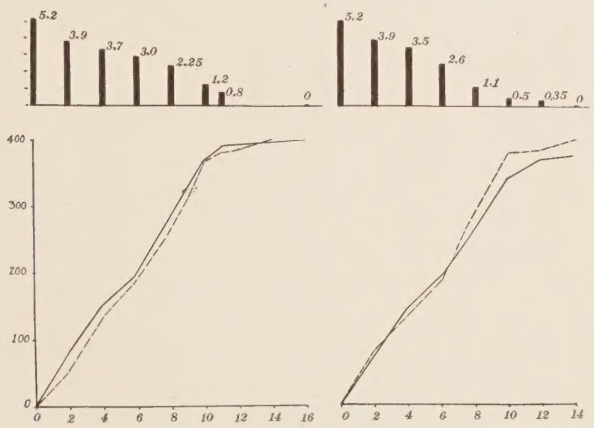
In the previous section it has been shown that Ascaris eggs develop through all stages at a proportional rate at 21°C. and 30°C. The next point to consider is whether or not oxygen consumption during embry-



Graph 3.—Experiments 1 and 2, development of eggs at 21 C.; solid line, 288,000 eggs per flask; broken line, 576,000 eggs per flask. Bar diagram gives consumption of oxygen per liter by eggs. Curves for control cultures, oxygen saturated at all times, coincided with curve for exp. 1. Ordinate shows index value; abscissa, days of development.

onic development is equally regular or whether certain stages of development require more oxygen than others. There is also the question of whether the slower embryonic development which takes place at 21°C. consumes more oxygen than the more rapid development at 30°C. To answer these questions a number of experiments were performed in which a known number of undeveloped eggs were put in flasks with 250 cc. of tap water (plus 1-1000 formalin). The oxygen content of the solution at the beginning of the experiment was found by oxygen determinations on the contents of several of the flasks. Flasks in which the water was kept oxygen saturated by frequent agitation were used to develop the eggs used as controls, to give the normal rate of development.

Graph 3 (experiments 1 and 2) gives the curve of development of two different sets of these experiments cultured at 21°C. The full line is the curve of development of an experiment in which 288,000 eggs were present in each flask. It is seen that the development was quite regular throughout all the embryonic stages and in 25 days 95% of the eggs were embryonated. There was 5.32 cc. of oxygen per liter present in each flask at the beginning of the experiment. The solid bar diagram gives the amount of oxygen present per liter of water in the flasks on the different days of the experiment. Thus on the 12th day, when the embryonic development was about one half completed, the oxygen content had been reduced from 5.32 to 4.13 cc. per liter; and on the 25th day when embryonic development was completed, only 2.1 cc. of oxygen per liter remained. Hence 5.32—2.1 cc. or 3.22 cc. of oxygen per liter was consumed in the development of the eggs. Since only 250 cc. of



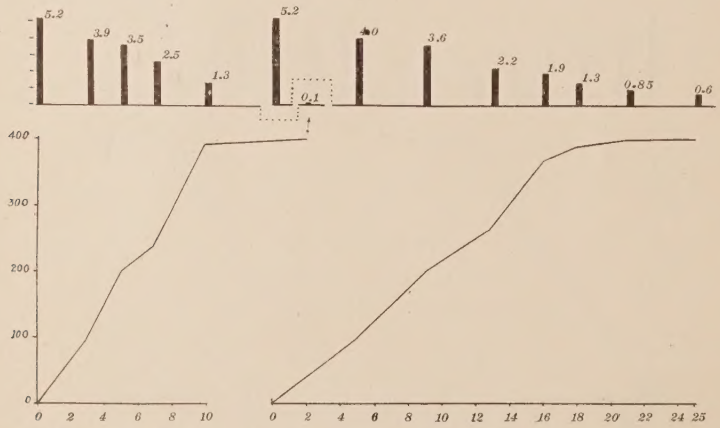
Graph 4.—Experiment 3 on left, 4 on the right. Development of eggs at 30 C. Solid line in exp. 3 shows 388,000 eggs per flask; 550,000 eggs per flask in exp. 4; broken lines, control culture O_2 oxygen saturated, at all times, about 5.0 cc. per liter. Ordinates show index value; abscissae, days of development.

water was present the eggs actually used 0.85 cc. of oxygen during their development.

The broken line gives the curve of embryonic development for flasks each containing 576,000 eggs. The broken line bar diagram gives the oxygen content of the flasks on different days. It will be seen from both of the bar diagrams that in general the oxygen was consumed at a fairly uniform rate. The development of the eggs in the control culture with oxygen saturation at times was exactly the same as the curve of experiment 1 and therefore was not put in separately in graph 3.

Graph 4 shows the same type of experiment (numbers 3 and 4) at 30°C. The rate of development of the eggs in experiment 3 with 388,000 eggs per flask, is shown by the full line and the oxygen con-

sumption by a bar diagram. During the 16 days of the experiment the oxygen consumed per liter is represented by the difference between 5.2 cc. and 0.05 cc. per liter the amounts present per liter, in the flasks on the first and last days of the experiment. Now if oxygen consumption by the eggs is grouped into quartiles of 4 days each it is found that the actual number of cc. of oxygen consumed in these quartiles is 0.375, 0.36, 0.36 and 0.2 cc., which except for the last quartile is very constant. A glance at graph 4 of development for this experiment shows that in the last quartile of development most of the eggs were completely embryonated, only about 15% completing their development during this time. Hence it is not surprising that oxygen consumption was low during this period as 85% of the eggs were embryonated and this stage uses up oxygen much more slowly than the rapidly developing stages. Experiment 4 is also shown on graph 4. This experiment also indicates



Graph 5.—Development of eggs in media gradually exhausted of its oxygen by the eggs; exp. 5 on the left shows 405,000 eggs per flask at 30 C., exp. 6 on the right the same number of eggs per flask at 23 C. Bar diagram shows cc. oxygen consumption per liter. Curves of development of eggs in control cultures, oxygen saturated at all times, coincide exactly with the curves of development of eggs in the flasks. Ordinates show index values; abscissae, days of development.

that oxygen is consumed at a fairly regular rate by the developing eggs, no stage of development consuming oxygen much faster than the others. About 550,000 eggs were present in each flask in this experiment and it will be seen from the graph and bar diagram that the eggs completely consumed all the oxygen from the flasks before all the eggs had developed completely. The significance of this will be taken up later.

To determine whether embryonic development consumes oxygen in the same amounts and at a proportional rate at the different temperatures an experiment was performed in which the eggs were incubated at 23° and 30°C. The eggs used in this experiment were obtained from the

anterior inch of the uteri of adult females. They were all developed to a complete one cell stage. This uniformity of the eggs to begin with, accounts for the extremely uniform development of the eggs during the experiment. Approximately 405,000 eggs were put in each flask and the flasks sealed. Approximately 5.2 cc. of oxygen per liter was present in the water of every flask at the beginning of the experiment. Graph 5 gives the curves of embryonic development of these two cultures. Development was exceedingly regular at both 23°C. and 30°C., 100% of the eggs becoming completely embryonated in 21 and 11 days respectively in the two cultures. Consumption of oxygen (bar diagram graph 5) was also very regular. To compare the rate of oxygen consumption at the two temperatures is a comparatively simple matter. The "index value" gives a number which is indicative of the total development of the eggs up to that time. Hence if one compares the oxygen left in the flasks of these two sets of cultures on days when their index values are identical or nearly identical, the oxygen consumed in development to that stage is obtained directly. Table 1 furnishes these data. Examination of this table shows immediately that the cultures at 23°C. and 30°C. in developing to any particular stage, as measured by the index value, consumed approximately the same amounts of oxygen. For example, when both cultures contained 100% of their eggs in the late morula stage (index value 200) the amounts of oxygen consumed by the eggs in reaching this stage were 0.4 cc. in the 23°C. culture and 0.43 cc. in the 30°C. culture. The length of time it took to consume these amounts of oxygen was 9 and 5 days respectively. Likewise it was found that when both cultures had index values of 392 both had consumed 0.97 cc. of oxygen. Since an index value of 400 indicates complete development of all the eggs it is seen that these two cultures had practically completed their development and in doing so had consumed identical amounts of oxygen, in one culture in 18 days and the other in 10 days. From the foregoing data it is seen that Ascaris eggs consume, in their embryonic development, the same amount of oxygen whether their development takes place at 23°C. or at 30°C. Also the rate of oxygen consumption is proportional to the rate of development of the eggs. That is, eggs developing rapidly at 30°C. consume oxygen more rapidly but the total used is no greater than at 21°C. or 23°C. On the 10th day of the 30°C. (table 1) culture and the 18th day of the 23°C. culture, 92% of the eggs had become completely embryonated (index value 392). Any further oxygen consumption was due mainly to that used by the motile embryos as at this time only 8% of the eggs were in the tadpole stage. Graph 5 shows that in six days the motile embryos at 30°C. consumed 0.3 cc. of oxygen while in seven days the same stage at 23°C. consumed only 0.18 cc. of oxygen. At this time both cultures showed 100% of the eggs to be completely embryonated. The embryos

in the eggs of the 30°C. culture, however, appeared to be more completely developed than those of the 23°C. culture and likewise the reserve food granules were much smaller and fewer. These data indicate that just as the developing stages use up oxygen at a faster rate at 30°C. than at 23°C., so the motile embryo consumes it at a faster rate at the higher temperature. No doubt the increased activity of the embryos at 30°C. accounts for the more rapid use of its food granules and the oxygen necessary to make them available for energy requirements.

The above experiments show that oxygen is consumed at a regular rate in the embryonic development of Ascaris eggs. No one stage of development consumes appreciably more oxygen than another. Oxygen

TABLE 1.—*Rate of Consumption of Oxygen by Ascaris Eggs Developing at 23 and 30°C.*

Stages of Development	Index Value	23°C		30°C		Cc. of Oxygen Consumed in Development	
		Days of Development	Oxygen (Cc. per Liter) in Flask	Days of Development	Oxygen (Cc. per Liter) in Flask	23°C	30°C
All one stage	0	0	5.2	0	5.2	0	0
All early morula	100	5	4.0	3	3.9	0.3	0.33
All late morula	200	9	3.6	5	3.5	0.4	0.43
Late morula and tadpole	240	11	?	7	2.5	?	0.67
Late morula and tadpole	265	13	2.2	8	?	0.75	?
92% with motile embryos	392	18	1.3	10	1.3	0.97	0.97
All embryonated	400	21	0.85	11	0.85	1.1	1.1

TABLE 2.—*Amount of Oxygen Consumed Per Egg in Development at 21, 23, and 30°C.*

Experiment Number	Graph Number	No. Eggs per Flask	Temperature	Total Cc. Oxygen Consumed	Cc. Oxygen Consumed per Egg
1	3	288,000	21°C	0.84	.0000029
12	.	375,000	21°C	0.95	.0000025
2	3	576,000	21°C	1.44	.0000025
5	5	405,000	23°C	1.09	.0000027
3	4	388,000	30°C	1.2	.0000031
6	5	405,000	30°C	1.09	.0000027

consumption at both 23°C. and 30°C. is equally regular in rate and the same amount is used for complete development. Motile embryos do not consume oxygen as rapidly as do the developing embryos, which in general agrees with the results of Fauré-Fremiet. He found *A. megalocephala* eggs to consume oxygen at a regular rate until embryonic development was about complete at which time the rate of oxygen consumption was markedly decreased.

AMOUNT OF OXYGEN CONSUMED BY A SINGLE ASCARIS EGG IN DEVELOPING TO THE EMBRYONIC STAGE

The amount of oxygen consumed by a single Ascaris egg in developing from the one cell to motile embryo stage is easily computed from the data given in the previous section. It is merely a question of dividing

the total oxygen consumed from the medium in the flask by the number of eggs present. Table 2 gives these data for six experiments. A glance at this table shows that the amount of oxygen consumed by a single egg in developing completely is indeed very small amounting to 0.0000025 to 0.0000031 cc. It will be noted that the eggs at 30°C. appear to consume slightly more oxygen than at the two lower temperatures. In view of the slight variations involved in oxygen determination and in estimating the number of eggs present in the flask it is very likely that this difference is due to experimental inaccuracies and that the figure of 0.0000025 cc. is approximately the amount of oxygen consumed by the eggs in completely developing at all three temperatures.

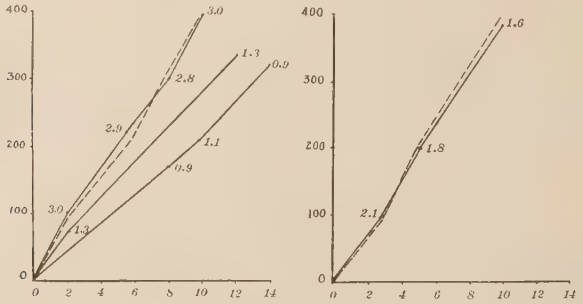
THE EFFECT OF REDUCED OXYGEN TENSION ON THE RATE OF DEVELOPMENT

If the normal rate of development of Ascaris eggs is that which occurs in an aqueous medium saturated with atmospheric oxygen, it becomes important to inquire into their rate of development when oxygen tensions are below those of saturation. The question at once arises as to whether there is a definite oxygen tension below which no embryonic development will take place, or will the development proceed at the low oxygen tensions, but at a reduced rate. It will be noted in experiment 2 (graph 3) that the 576,000 eggs per flask used up the oxygen so that when development was about three quarters finished (index value 300), the oxygen in the flask was between 2.1 and 1.1 cc. per liter. From this point on the eggs to develop from index value 300 to 395 in the control cultures consumed 9 days while experiment 2 consumed 14 days (graph 3). Likewise, graph 4 of experiment 4, shows that when the oxygen in the flasks became about 1.1 cc. per liter, about $\frac{1}{5}$ saturation, the rate of development of the eggs was slowed down considerably. The oxygen in the flasks became used up and when completely gone development stopped. It will be seen also (graph 4, experiment 4) that the lower the oxygen tension became below 1.1 cc. per liter, the slower was the development of the eggs. The curves of these experiments and their oxygen diagrams show that no slackening in embryonic development is noticeable when the oxygen tension in the flasks had been practically cut in half (2.6 to 2.1 cc. per liter). These two experiments indicate very clearly that, at least for the final phases of embryonic development, the oxygen tension necessary is much below that afforded by water saturated with atmospheric oxygen at 30°C. It becomes pertinent to ascertain whether or not the early stages of embryonic development react in the same manner to reduced oxygen tensions. To this end a series of experiments was carried out in which the eggs were incubated in water partially freed of its dissolved oxygen. By means of boiling the water, the oxygen was driven out and a series of flasks containing 3.0, 1.8, 1.3

and 0.9 cc. of oxygen per liter were obtained. These were sealed in the usual way and when cool 40,000 undeveloped Ascaris eggs were put in each flask and the flask immediately resealed. This number of eggs does not consume sufficient quantities of oxygen during development to diminish appreciably the total amount in a flask. Half of these cultures were then incubated at 23°C. and 30°C. respectively.

It will be seen (graph 6, experiments 7 and 8) that the development of the eggs at 30°C. in the flasks in which the oxygen had been reduced to approximately 3.0 cc. and 1.8 cc. of oxygen per liter was perfectly normal. The small numbers along the curves give the oxygen present per liter of water in the flask. The curves of development of the eggs in these reduced oxygen tensions are practically identical to those of cultures having oxygen saturation all through development, as shown by the broken line curves.

The rate of development of the eggs in water at 30°C. with only 1.3 and 0.9 cc. of oxygen per liter is much slower than normal develop-



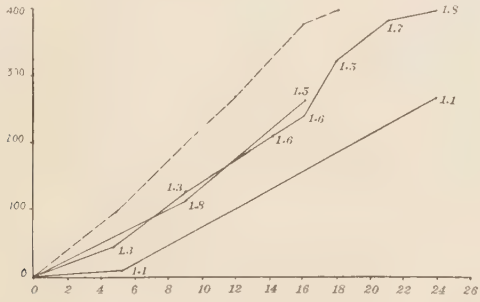
Graph 6.—Experiment 7 at left, retardation of developing eggs in media low in oxygen, at 30 C. Experiment 8 at right, development of eggs at low oxygen tensions, at 30 C. In both experiments solid lines show rate of development. Oxygen as cc. per liter in these experiments shown by numbers on graph lines. Broken lines show control O_2 saturated; ordinates, index value; abscissae, days of development.

ment (graph 6, experiment 7). It will be seen that when the control culture contained 100% of its eggs embryonated, index value 400, that the culture with 1.3 cc. oxygen per liter had an index value of 285. This indicates a 30% retardation of development up to this point. Likewise on this same day the index value of the culture with approximately 0.9 cc. of oxygen per liter was 200, showing that its development was retarded 50% up to this point.

Graph 7 gives the curves of development of eggs at 23°C. in oxygen tensions of 1.8, 1.5 and 1.1 cc. per liter. It will be seen that the rates of development in oxygen tensions from 1.3 to 1.8 cc. per liter are indistinguishable as far as their retarding effects on the development of the eggs are concerned. In general the development of the eggs at these

oxygen tensions is retarded about 30% as compared to the curve of development (broken line) of eggs in oxygen saturated media. Likewise the eggs at an oxygen tension of 1.1 cc. per liter are slowed up in their development about 50%. In general these retardations are the same as found under similar oxygen tensions at 30°C. except that at this temperature (30°C.) no retardation was found in media of 1.8 cc. oxygen per liter, while this oxygen tension at 23°C. resulted in a 30% retardation of development. It has been suggested that the reason that the eggs of *A. suum* do not develop at 37°C. might be due to lack of sufficient oxygen, as at this temperature only 4.5 cc. per liter is soluble. The above experiments, however, indicate that it is not lack of oxygen that prevents their development at 37°C.

The question of whether Ascaris eggs can utilize oxygen at low tensions and consume completely the oxygen present is answered in experiments 1, 2, 4, 10 and 11 (graphs 3, 4 and 8). Graph 3 shows the oxygen in the flasks to have been consumed down to the point where

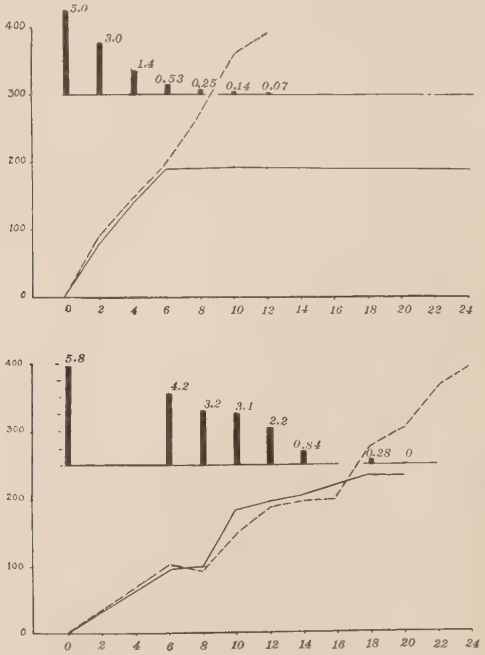


Graph 7.—Experiment 9. Retardation of developing eggs in media low in oxygen at 23 C. Solid lines show rate of development. Oxygen as cc. per liter shown by numbers on graph line. Broken lines, control O_2 saturated, 6 cc. per liter. Ordinate shows index value; abscissae, days of development.

only 0.2 and 0.1 cc. per liter remained. Graph 4 shows the oxygen in the flasks of experiment 4 to be completely exhausted on the 14th day of the culture. In all three of these experiments the motile embryo state of embryonic development was present when the oxygen was so reduced. In two experiments (10 and 11) in which 1,300,000 and 830,000 eggs per flask were used the oxygen was completely exhausted by the time the eggs were in late morula and tadpole stages respectively (graph 8). These experiments proved that not only the motile embryo but also the late morula and tadpole stages of embryonic development can utilize oxygen from water at extremely low tensions and can completely exhaust water of this gas. As noted before, however, development at these extremely low oxygen tensions is greatly slowed up, and stops as soon as the oxygen is entirely used up.

THE EFFECT OF OXYGEN PRESSURES ON DEVELOPMENT OF ASCARIS EGGS

Fauré-Fremiet's (1913) contention that the embryonic development of Ascaris eggs depends upon oxidation of their reserve carbohydrates and fats brings up the question of whether or not this oxidation, and therefore the rate of development, can be hastened by means of high oxygen tensions. An attempt was made to answer this question in the following experiments. Into each of two 250 cc. flasks were placed 100 cc. of tap water plus formalin, and 160,000 eggs in the one cell stage.



Graph 8.—Complete exhaustion of oxygen from media by eggs in the late Morula and tadpole stages of development. Experiment 10 above. Oxygen consumption by 1,300,000 eggs per flask (solid line) at 30 C. Broken line shows control O_2 saturated. Experiment 11, below. Oxygen consumption by 830,000 eggs per flask (solid line) at 21 C. Broken line control O_2 saturated. Bar diagram shows cc. oxygen consumption per liter. Ordinates show index values; abscissae, days of development.

The one flask was then corked and the side-arm connected to an air pressure hose. An air pressure of 506 mm. (10 pounds) was then put upon the fluid in the flask and the side-arm of the flask clamped off. The contents of the flask were then under a total air pressure of 25 pounds, 15 pounds atmospheric plus the additional 10 pounds air pressure. As a result about 8.5 cc. of oxygen per liter of water was present

in the culture solution. The second flask was connected to an oxygen tank and after oxygen had been forced through the flask displacing the air above the fluid it was stoppered and the contents put under 10 pounds oxygen pressure. The two flasks were placed in a 30°C. incubator along with a control under normal atmospheric pressure and oxygen content. The flasks were shaken daily to keep their fluid oxygen saturated and at 3 day interval they were uncorked and a number of eggs examined as to stage of development. The flasks were then recorked and put under pressure again and placed in the incubator. At the end of 10 days practically 100% of the eggs in the control culture contained motile embryos. Likewise about 100% of the eggs under the 10 pounds additional air pressure contained motile embryos. The rate of development in this culture was exactly the same as the control culture despite the fact that medium contained nearly twice as much oxygen per liter as the control culture. The eggs in the flask under 10 pounds additional oxygen pressure were in a medium containing about 21 cc. of oxygen per liter. These eggs developed to the two and four cell stages and then ceased to develop. These experiments indicate that additional oxygen, above that found in water oxygen saturated from the atmosphere, does not hasten the rate of development of Ascaris eggs. This is also indicated in the experiments in which the eggs developed in a medium with greatly reduced oxygen. The fact that the eggs in both experimental flasks were under an additional 10 pounds pressure indicates that it was not the pressure alone that was the lethal factor. It appears rather that the specific oxygen pressure prevented the development of the eggs and also caused their death.

DISCUSSION

The results of the experiments recorded above indicate that some of the prevalent conceptions on oxygen utilization by Ascaris eggs are not well founded. The fact that in stoppered bottles filled with water partially exhausted of its oxygen, Ascaris eggs did develop indicates that a medium far from saturated with oxygen permits their development. Hence the experiments by Ransom and Foster (1920) in which eggs put in stoppered bottles filled with water failed to develop, do not indicate that the water did not originally contain sufficient oxygen for development. Rather, this experiment indicates that the bacteria and organic matter present consumed all the oxygen before the eggs were able to begin their development. Likewise it has been noted by several authors that the stirring of petri-dish egg cultures, every few days, hastens their development. This is a well known fact, but rather than by completely saturating or even supersaturating the water with oxygen as some suggest, it probably only raises the oxygen content of water, in which oxygen has in some way been reduced, to the tension at which develop-

ment can continue. The studies outlined in this paper show that supersaturation of water with oxygen does not hasten egg development.

The amount of oxygen necessary for the complete development of Ascaris eggs has been ascertained by Fauré-Fremiet (1913:615) for the eggs of *A. megalocephala*. He found that 1 gram of eggs, dry weight, in completely developing used 50 cc. of oxygen. This, however, gives no adequate conception of the amount used by a single egg since the number of eggs per gram of weight is not known. He also found that the motile embryo used less oxygen than the developmental stages which in general checks the results along this line in this study. Wharton (1915) reported that the total amount of oxygen consumed by *A. suum* eggs in completely developing was very slight as he found that two eggs developed completely in a single drop of water sealed in a hanging-drop slide. Using the figures of 0.3 cc. as a drop of water and 6 cc. oxygen per liter as its original saturation the amount of oxygen present in a drop is found to be 0.0018 cc. which was ample to allow complete development of the two eggs. In fact, from the amount of oxygen found in this study to be necessary to completely develop one Ascaris egg, it is apparent that such a drop of water would contain sufficient oxygen to develop completely about 300 eggs.

It is interesting to examine the results of the experiments on oxygen requirements of Ascaris eggs in the light of the oxygen supply they normally encounter in nature. Eggs deposited in stools which are gradually broken down by bacterial growth are probably surrounded by a medium rather low in oxygen due to bacterial activities. Once the feces are consumed by bacteria and insects and the remaining debris and eggs spread over the soil surface the moisture surrounding them is probably nearly saturated with oxygen. However, in humus soils, and this is probably partially true in other types of soil, if the eggs are washed below the upper layer and forced below the surface they are quite likely to be surrounded by bacterial growth and hence a lessened oxygen tension. Hence it is quite likely that Ascaris eggs have become adapted to developing in nature in a medium which was not fully oxygen saturated with the result that the higher oxygen tensions are not necessary for normal development.

It has been suggested by a number of workers that infestations of Ascaris may arise from drinking water from shallow pools previously polluted with human excrement. It is therefore important to know whether the amount of oxygen encountered by the eggs in the bottom of such pools would be sufficient for development. Manifestly, if the eggs sink into a mud bottom devoid of oxygen, development could not take place and since, three months in an oxygen free medium is all the eggs can remain viable, infestations from this sort of pool could not take place. Pools in nature without oxygen in their bottom water are

numerous, likewise pools with heavy aquatic growths on their bottoms are often found to have abundant oxygen in their lower waters due to photosynthetic processes. That Ascaris eggs do not find sufficient amounts of oxygen in a pool does not, however, eliminate the possibility of completely embryonated eggs being washed into a pool. In this way an infestation might arise from a pool which did not have sufficient oxygen in its bottom water to permit development of eggs. Experiments by the writer indicate that fully embryonated Ascaris eggs are not injured by exposure to oxygen free water for periods of six weeks or longer. It would be very interesting to determine in the field the oxygen content at the bottom of a series of pools likely to be contaminated with feces containing Ascaris eggs. It would also be of interest to study the oxygen demands and rate of consumption of the species of Ascaris eggs such as the dog ascarids the development of which is much more rapid than the form used in this study.

CONCLUSIONS

1. A detailed quantitative study is presented of the influence of temperature and oxygen upon the embryonic development of the eggs of the *Ascaris suum* from the pig. Incubation of the eggs was in 1-1000 formalin-tapwater medium.

2. Development is very regular at both 21°C. and 30°C. in media saturated with atmospheric oxygen, approximately 6.4 and 5.2 cc. of oxygen per liter respectively.

3. Development is 2½ times as rapid at 30°C. as at 21°C.. Each stage of development is hastened equally by this 9°C. raise in temperature.

4. Oxygen consumption by the developing eggs is very regular; no one stage consumes more than any other stage.

5. Oxygen consumption by eggs developing at 21°C. is slower than that of eggs developing at 30°C. due to the slower development of the eggs at the lower temperature. The amount of oxygen consumed by eggs to develop to any given stage at these two temperatures is identical.

6. Completely embryonated eggs consume oxygen more slowly than developing eggs and the consumption of oxygen by embryonated eggs at 30°C. is nearly twice as rapid as that of eggs at 21°C.

7. A single Ascaris egg consumes about 0.0000025 cc. of oxygen during its development.

8. Eggs in the morula, tadpole and embryonated stages can completely exhaust oxygen from their culture medium.

9. Embryonic development proceeds normally in media deprived of as much as one half of the oxygen found in water saturated with atmospheric oxygen at 30°C.

10. In media with approximately 1.3 to 1.8 cc. of oxygen per liter at 23°C., development is retarded about 30%. At this same temperature the embryonic development is retarded 50% in media with about 1.1 cc. of oxygen per liter.

11. At 30°C. media with 1.8 to 3.0 cc. oxygen per liter permit development at a normal rate while in media with 1.3 cc. and 0.9 c. oxygen per liter development is slowed down approximately 30% and 50% respectively.

12. Oxygen pressures do not hasten embryonic development and when sufficiently great (506 mm.) prove lethal to the developing embryo in the very early stages of development.

Literature Cited

American Public Health Association. 1923.—Standard Methods of Water Analysis. 5th ed., pp. 59-61.

Fauré-Fremiet, M. E. 1913.—Le cycle germinatif chez *l'Ascaris megalocephala*. Arch. anat. micr., 15:437-736.

Hallez, P. 1885.—Recherches sur l'embryogénie et sur les conditions du développement du quelques nématodes. Mem. soc. sci. de Lille, 15:1-71.

Martin, A. 1913.—Recherches sur les conditions du développement embryonnaire des nématodes parasites, Ann. sci. nat., zool. 9 ser., 18:1-151.

Ransom, B. H. and Foster, W. D. 1920.—Observations on the life history of *Ascaris lumbricoides*. Bull. U. S. Dept. Ag., no. 817.

Wharton, L. D. 1915.—The development of the eggs of *Ascaris lumbricoides*. Phili. Jour. Sci., B. 10:19-25.

Wigdor, M. 1918.—Some studies on the resistance of the ova of *Toxascaris limbata*. New. Orl. Med. Surg. Jour., 71:264-281.

Zavadovsky, M. 1916.—Le développement des oeufs *d'Ascaris megalocephala* dans un milieu putréfié. C. R. soc. biol., 75:798-802.

A STUDY OF EXCYSTATION IN *NYCTOTHERUS OVALIS*

WITH NOTES ON OTHER INTESTINAL PROTOZOA OF THE COCKROACH

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The main purpose of the present paper is to give an account of the development of the ciliate *Nyctotherus ovalis* from its resting phase, and of its final emergence from the cyst wall as a free-swimming trophozoite. The process was initiated experimentally by feeding the host with cysts and was then watched throughout under the microscope in a fresh preparation of the midgut contents. Attempts were also made to test the efficacy of certain environmental factors in stimulating the organism to excyst. Of those so far tried, a rise of temperature to 28 to 30° C. has proved successful.

The remaining subject matter deals mainly with a few observations on the other Protozoa of the cockroach, made during experiments on *Endamoeba blattae*. The work on the latter form was carried out with a view to confirming or modifying the conclusions reached by Mercier (1910). The experiments, however, impeded by extreme scarcity of material, gave only negative results. They will be briefly summarized in a later paragraph.

The observations on excystation in *N. ovalis* seem of interest, since nothing has been done up to the present on this phase in the life of a parasitic ciliate. Detailed accounts of the process as it occurs in free-living forms are very few, and only within the last two years has it been described in any parasitic Protozoon. Cutler (1919) apparently saw excystation in *Entamoeba histolytica,* but no account of it was published until that of Yorke and Adams in 1926. In *Iodamoeba bütschli* the process was completely described by Smith (1927). A similar study was made of *E. coli* and *Endolimax nana* by Hegner (1927) and certain observations were made by the same writer on excystation in *Chilomastix mesnili* and *Giardia intestinalis.*

In all the above cases the phenomenon occurred in parasites of man and was induced artificially *in vitro.* As far as has been ascertained, the excystation of a Protozoon in a lower animal has not hitherto been described; nor have the conditions affecting excystation in a cold-blooded host been analyzed. Furthermore the relative complexity of structure in such a specialized form as *Nyctotherus ovalis* lends particular interest to the manner of its reorganization.

The work on *Endamoeba blattae* (Bütschli, 1878) Leidy, 1879 was begun at the London School of Tropical Medicine, and the material used there consisted of *Blatta orientalis*. This, the common black cockroach, was obtainable from the kitchens of certain large institutions in the city, and two out of the three colonies examined were infected with the amoeba. The single observation on excystation in *Lophomonas striata* Bütschli, which is recorded below, was also made at this time.

The remainder of the work was done at the Johns Hopkins University, and here the large cockroach *Periplaneta americana* was used. This contains an intestinal fauna closely similar to that of *B. orientalis* and is in some respects more favorable material to work with. The cockroaches were obtained from four different colonies in Baltimore, and though specially plentiful later in the year, were to be had all through the winter. Infections with *Nyctotherus ovalis* Leidy were heavy and occurred in almost 100% of individuals from all colonies. Three colonies were infected with *Lophomonas striata* and two with *Endamoeba blattae*. A consignment of *Periplaneta australasiae* from Florida has also been used in the work on Nyctotherus for the sake of comparison. There again the ciliate was very prevalent.

A difficulty, however, throughout the work, was the scarcity in nature of the cysts of all the cockroach Protozoa. This may be correlated with the fact that several circumstances combine to make transmission of infection a simple matter in other respects. The cockroaches live in crowded quarters, their duration of life is relatively long, and when in need of water they will consume fecal matter readily. The method used to obtain cysts of either *E. blattae* or *N. ovalis* was to isolate each cockroach obtained from the infected colonies, as soon as it was caught. If the feces proved positive for cysts the insect was kept in isolation as a source of supply. In spite of the very high incidence of infection with *N. ovalis*, yet cysts were revealed in the feces, after two thorough examinations, in only 4% of individuals. The cysts were often passed for only a few days, and frequently there were only half a dozen or less in each fecal specimen.

Desiccation, as complete as would be likely to occur in nature, causes shrinkage and apparently death in the encysted organism. It was therefore important to keep the feces moist, for they were usually passed at night and could not be used immediately. The insects were accordingly kept each in a glass jar provided with wire gauze lid, and the bottom lined with layers of damp filter paper.

EXCYSTATION IN *Nyctotherus ovalis* IN VIVO

In order to induce excystation *in vivo*, cockroaches were made to consume feces containing *N. ovalis* cysts, and the contents of the alimentary canal were afterward examined in the fresh state. Before

these experiments could be done, it was necessary to determine roughly the time taken for a meal to pass down the alimentary tract. Starved specimens of *P. americana* were accordingly fed a meal consisting of moist bread mixed with carmine and were dissected, a few at a time, at intervals of about 6 hours. This revealed a high degree of diversity between different individuals, comparable to that found by Sanford (1918), and by other workers on the genus. It may take from 6 to over 48 hours for an appreciable amount of the meal to pass from the crop into the midgut or stomach. It was this transition which was of greatest importance in the experimental work, since excystation was expected to occur in the midgut. This was where Mercier found it in *E. blattae,* and it is in this organ that the main part of digestion occurs. Moreover, waste products pass from the midgut directly into the hindgut, where nearly all the intestinal Protozoa are to be found. To determine the average length of the critical period, test experiments were repeated, and dissections made at hourly intervals after the first 5 hours. The position of food in the midgut was indicated on a graph in each case, and the curve thus obtained suggested that the most probable time for the organ to become filled was about 16 hours. The average time in *B. orientalis,* though it has not been accurately determined, seems to be several hours less. In *P. americana* the stomach would not be entirely emptied in less than 24 hours, and since the crop acts as a storage organ, might be continuously refilled for days. In the experiments, however, the meal was not large enough for this to be the case.

These data obtained, cockroaches which had been starved for from 2 to 7 days were fed with fresh infective feces procured as outlined above. Since the supply of cyst-carriers was generally limited to 2 or 3 at a time and most of the cysts degenerated on keeping, methods of concentrating these were not practicable. No difficulty was found in inducing the insects to eat bread-crumbs soaked in the feces diluted with 0.5% saline. If sufficiently long starved, they required no bait at all, which facilitated the search for cysts, afterward made in the intestinal contents. After intervals varying from 10 to 20 hours, the cockroaches were anaesthetized and dissected, and some or all of the meal usually found in the midgut. The contents of this organ, and in certain cases of the crop or hindgut also, were examined as a smear in 0.5% saline. The coverslip was sealed, and the grit always present in the feces prevented its pressing on the cysts. The organisms, when found, were watched under oil immersion lens at magnifications of x 1200 or x 1900. No stained preparations were made, for even boiling Grenacher's carmine only slightly penetrates the cyst wall of *N. ovalis.* Thus nuclear changes, if any, could not be followed. Section-cutting would have

proved too laborious a method, since examples of excystation occurred few at a time, and in only a small proportion of the experimental animals.

The structure of a ripe *N. ovalis* cyst has been briefly described in an earlier paper (Lucas, 1927) *, and is shown in figure 1. The wall, which at first appears homogeneous, proves under high magnification to consist of three layers. The outer (not shown) is a thin, hyaline and colorless shell, the ectocyst. It is almost, or quite, indistinguishable, unless it is injured and begins to scale off. The knob-like process of the wall at one end of the cyst is mainly formed by a thickening of the central layer, which is elsewhere very thin. The inner layer, on the other hand, contributes almost the whole thickness of the entire cyst wall, except around the base of, and within, the knob. The two inner layers combine to form a tough endocyst which is yellowish in color. Lining the inside of the wall is a thin membrane, or intimocyst, which is scarcely visible except when the cyst is empty (shown only in Fig. 6). The end of the cyst which bears the knob represents the posterior region of the encysted organism. The cytoplasm in freshly formed cysts shows surface striae representing the former ciliary grooves. The macronucleus is slightly anteriorly placed, but not as far forward as in the free form. The neighboring micronucleus can only rarely be detected, since it appears to be a small, perfectly hyaline sphere, and is easily obscured. Around the macronucleus is diffused a quantity of characteristic granules, derived from the compact mass which occupies the anterior end of the trophozoite. When the encysting ciliate contains "elliptical or spindle-shaped bodies" (probably also reserve food), these are aggregated in the cyst at the blunt end (Fig. 1). Irregular bodies of a similar appearance sometimes occur in this position in other examples.

The organism is thus nearly, but not completely, dedifferentiated in the cyst. The original polarity is indicated by the position of the nucleus and by the surface striations of the cytoplasm. There are no traces of organelles.

A variable time elapses before the cysts consumed by the host will show visible signs of approaching excystation. Individual cysts evidently differ. Probably about 11 hours is normal, but various stages may be found simultaneously. The usual scene of action, as was expected, is the midgut or stomach, and the process has not been seen to occur in the crop. It is not proved, however, that the environmental conditions of the midgut are a *sine qua non*. On one occasion a form in the preparatory stage was found in the hindgut 24 hours after ingestion, but whether the process could have been completed in this locality is uncertain.

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* In the paper referred to, the magnification of figures showing *N. ovalis* cysts was not correctly specified. It was × 750.

Only an estimate can be made of the average time taken for excystation, including preliminary stages, to be completed on the slide. The organism sometimes dies in these conditions before entirely emerging, and the process can rarely be traced from beginning to end in the same individual. In cases observed it has taken from 6 to 8 hours, probably a little longer in the unfavorable environment than in nature. According to evidence given above, undigested food matter would begin to pass out of the midgut on average about 16 hours after ingestion. The ciliate is therefore generally carried into the hindgut a short time after emerging; but in many cases where digestion is slow, infection must fail to be set up unless the trophozoite can withstand conditions in the midgut for some hours. That it is able to do so is suggested by the survival of the newly excysted individuals in the smear for several hours; also by the finding of free forms in the midgut of a cockroach on two occasions in nature.

The first change to be seen in the cyst is in the central cytoplasm, which appears to be fluid (solated) and begins to show a slight indefinite flowing movement. The striated layer on the surface is not at first involved in this streaming. The movement increases however in activity, and when spindle-shaped bodies are present, they become diffused throughout the endoplasm so that the latter comes to appear the same at both poles.

Development and reorientation then begin. The organism develops with reversed polarity, so that it ultimately emerges from the knobbed, originally posterior end of the cyst wall, with its anterior end foremost. After 1 to 2 hours the nucleus, moved by the stirring of the cytoplasm, has come to lie near the knobbed end. The small food granules gradually aggregate beyond the nucleus, and by the time the ciliate emerges they will have formed a compact mass (Fig. 7). This is relatively small compared with that of the adult, and it seems not unlikely that the food reserve is partially used up during development. The situation of the future cytopharynx and peristome becomes evident as a streak, which is gradually cleared of coarser granules, and finally becomes a fluid-filled narrow tube within the cytoplasm. Figure 2 represents the earliest stage of differentiation. The original surface striations, which have faded, next disappear completely.

Cyclosis then subsides, and the first ciliary action becomes noticeable. This is not at the surface but at the base of the cytopharynx. Here a faint undulating movement is seen, but the thickness of the organism makes its exact nature impossible to determine. Very soon afterward a continuous row of transverse striations gradually becomes clear, running backward from the anterior end of the body down the peristome, and then inward along the antero-dorsal wall of the cytopharynx (Figs. 3, 4). In sectional view this striated surface proves to be grooved, like the

periplast of the free form, and in each groove a membranelle ultimately forms. The fore part of the row of striations is thus the "adoral zone" which runs along the ventral (left) side of the peristome in the fully-formed ciliate. The peristome—later a groove in the right side of the organism—at present seems to be only slightly, if at all depressed and appears to extend only a short distance backward. The mouth at its posterior end is indistinguishable until shortly before the ciliate emerges and is probably closed at this time. Rhythmic movement is soon to be seen all along the row of striations. The action may be due to free cilia, for although these cannot be made out very distinctly, no membranelles can be detected. The latter are first visible just before the ciliate emerges and are then well-marked. Possibly they not only correspond to, but originate as, rows of cilia.

Striae, representing the ciliary grooves, are soon visible once more on the surface of the organism. It is interesting to note that each of the membranelle-bases of the adoral zone is directly continuous with one of the striae of the ventral surface (Fig. 5). The body gradually retracts a little from the cyst wall, and at the same time the surface cilia begin to develop. They become progressively more distinct, but whether by lengthening or increasing in number could not be determined.

About one-half hour after the first appearance of surface cilia, or 3 hours from the beginning of excystation, their development appears to be complete, and the whole organism commences revolving slowly about its long axis. At about this time a vacuole forms close to the posterior end and increases in size until it collapses into the now distinct cytopyge. The contractile vacuole is thus established, its rhythm being irregular and slow at first. At this stage one or two extraneous vacuoles sometimes exist simultaneously. Differentiation is apparently as yet imperfect; but by the end of another hour the contractile vacuole is functioning alone and pulsating regularly at intervals of about 1.5 minutes. The body by this time occupies less space within the cyst and is surrounded by fluid. Anterior to the nucleus a slight clearing in the cytoplasm indicates the position of one of the two transverse "septa." The membranelles are now distinct.

The ciliate, thus equipped with the necessary organelles is ready to emerge from the cyst wall. A sudden rupture occurs at the junction of the knob and the rest of the wall, and the knob itself is pushed off by internal pressure like the operculum of certain Trematode eggs (Fig. 6). By ciliary action the organism forces its way out through the narrow opening with a screw-like movement. It leaves the cyst wall intact, after taking perhaps an hour to emerge. The ciliate (Fig. 7) differs from the typical trophozoite in certain respects. It is of course much smaller in size and has a nucleus large in proportion. The cytoplasm is more transparent, having no inclusions, the granular

body is smaller and less compact, the anterior "septum" is absent, and the posterior one is very indefinite. The peristomial groove is shallow. Moreover, it is scarcely wider than the adoral zone of membranelles, though the latter will occupy only the ventral edge of the definitive peristome. Finally the cytopharynx does not yet show the length and backward curvature characteristic of it in this species. In short, the organism is distinctly less specialized than the fully-formed trophozoite. Newly excysted individuals have been kept alive on the slide between 12 and 24 hours.

EXPERIMENTS TO FIND FACTORS INDUCING EXCYSTATION

Experiments were directed toward determining the effect of certain environmental factors on the cysts. It was hoped in particular that the stimuli bringing about excystation in nature might be deduced, but no success has yet been met with in this direction. Owing, however, to scarcity of material, the experiments could only be performed a few times each, and the results obtained are not therefore conclusive. A method of inducing the whole process artificially was found; but this involved the stimulus of heat.

Endosmosis seemed a factor which might be involved. The entry of water by this means might have the same stimulating effect as imbibition of water has on free-living cysts, or might at least cause bursting of the cyst wall. It seemed not impossible that this factor operated through the osmotic pressure of the midgut-content being less than that of the hindgut. An attempt was therefore made to determine whether endosmosis could be artificially produced. Tests on the free forms indicated that a sodium chloride solution isotonic to the ciliates contained between 0.5% and 0.7% salt. A 0.5% solution was used to dilute feces slightly, and individual cysts were picked out under the microscope with a fine pipette so that little fluid was carried over with them. They were placed, some in 0.3% sodium chloride, and some in distilled water, in hanging drops. In neither case was there any change after 24 hours. Apparently then, if endosmosis does occur, it alone has no appreciable effect on the organism.

The obvious possibility presents itself that the chemical nature of the stomach contents act in some way as a stimulus to the cyst, though they do not appear to destroy the wall. This could not be proved, as will be seen below. To test the efficacy of digestive juices, cysts were picked out as above, and placed in hanging drops of three fluids respectively.

Gastric secretion of the cockroach was collected from the gastric ceca of a number of insects and used fresh. The ceca, or diverticula of the stomach, secrete the same fluid as the walls of the organ itself. According to Abbot (1926), they produce lipase, amylase, maltase, sucrase and trypsin. This secretion, he states, is responsible for the

whole process of digestion in the insect, including what occurs in the crop through regurgitation. Cysts exposed to the fluid showed no signs of excystation after 24 hours. Similar negative results have been obtained by other workers with *Endamoeba blattae* cysts. As would be expected from the *in vivo* experiments, the Nyctotherus cyst wall was unaffected. It is possible that it consists of a carbohydrate related to cellulose, a substance which the cockroach cannot digest. According to Goodey (1913) the endocyst of *Colpoda cucullus* is of carbohydrate, and among various enzymes he tried, only diastase would digest it.

The effect of human gastric and duodenal juices was tested for the sake of comparison. They were obtained from hospital patients under suitable conditions and used either perfectly fresh, or after keeping in an icebox not more than 12 hours. After filtering they were diluted with distilled water in the proportion 1 : 1. Cysts exposed to these fluids for 24 hours showed signs of nuclear degeneration, and were apparently dead. The walls were again unaffected.

Rise of temperature to about 28°C. proved to be the only stimulus successful under experimental conditions. This was a somewhat unexpected result. The cyst would not be subjected to such an influence on entering the host, unless it be that the digestive processes occurring in the stomach, result in a considerable evolution of heat.

Feces containing cysts were diluted with 0.5% saline, and placed on a slide with the coverslip sealed. The preparations were then studied on the microscopic stage, and this kept at the desired temperature by means of a 120-watt carbon filament lamp turned on above it. The globe, fitted with shade, is held by an adjustable clamp-stand, and a thermometer is supported with its bulb on the stage. By slightly raising or lowering the lamp, the temperature can be regulated with considerable accuracy. After the first 30 minutes it is found to vary as a rule less than $\pm$ 1° C. in several hours, if not exposed to noticeable draughts. Since the preparation is being watched more or less continuously, even this variation is easily prevented.

At 37° C. no movement occurred in the cytoplasm of the cysts, and vacuoles developed which did not contract; the wall did not rupture. The cyst contents finally shrank considerably and the organism appeared to be killed in every case. At 30°C. excystation was induced, and completed in a small proportion of the cysts exposed. It began between one-half and 1 hour after heat was applied and was on several occasions watched throughout. Often, however, at this temperature, the contractile vacuole, which appeared at the very beginning, would enlarge abnormally and the organism die at some stage in the process. Sometimes the operculum would be shot off explosively, even to a distance the length of the cyst itself. The pressure is evidently due in part to expansion of the cyst-contents under the physical effect of heat. But

it must also be partly caused by the life processes within, since it does not occur at higher temperatures when the cyst is dead.

The most suitable temperature for the experiment has proved to be 28° C. Excystation is then completed in the majority of viable and ripe cysts, and with only slight deviation from the normal. It is interesting to note that for *Colpoda cucullus,* Bodine (1923) reports 27° C. as the "optimum" temperature for excystation and anything above 36° C. as inhibitory. The only important irregularity occurring at 28° C. is one also encountered at 30° C. It is the formation at the commencement of the process of a contractile vacuole in the position of that of the precystic ciliate. The cytoplasm seems to retain some special quality at this point, for a vacuole forms there (probably from a different cause) even in nonviable cysts. In active forms it gives place later to the new contractile vacuole at the opposite end. In two examples of unripe cysts where there were still traces of the preceding cytopharynx and peristome, an anomalous effect was observed. The whole organism developed with the original orientation, the surface striae and the parts related to the mouth merely becoming more pronounced. The ciliate, reformed, was thus lying with its posterior end toward the operculum of the cyst. It did not emerge. At 26°C. early stages of excystation have occasionally been seen, but the process was not brought to a conclusion. At 25°C. there is no effect on the cysts.

When the source of heat is removed, shortly after development has been initiated by rise in temperature, excystation is not completed. This fact suggests that, under the conditions of the experiment, the higher temperature is not only a stimulus but a necessary environmental factor. How it brings about its effect remains unexplained. A temperature as high as 28°C. is not a necessary condition for excystation initiated *in vivo.* The latter has been found to proceed at room temperatures not over 23°C.

DISCUSSION

Certain features of the process of excystation in *N. ovalis* may be emphasized here, especially those bearing on more general problems. It is interesting that the contractile vacuole, under the more normal conditions provided by the first set of experiments, does not begin to operate when the excystation process starts. In *Lembus pusillus,* according to Hoare (1927), and in *Colpoda cucullus* according to Goodey (1913), and possibly in all the free living ciliates, the development of the vacuole is the first sign noticeable. The difference supports the theory that the vacuole in the free-living species is developed in the first place in connection with the imbibition of water that initiates excystation. In the Nyctotherus cyst which has not been desiccated, such absorption of water is probably absent or slight, and the development of the vacuole is delayed. Its first function would seem to be connected with elimi-

nation of the products of katabolism, since it begins to operate at the time when general movement of the body commences within the cyst. The appearance of the vacuole at this stage indicates the importance of those of its functions that are unconnected with digestion. The fact that it is formed prematurely under the influence of heat may be due to an increase in osmotic permeability.

The *N. ovalis* cyst is interesting in having a predetermined point of exit and a definite operculum. The experiments throw some light on the actual means by which the ciliate emerges. Clearly there is no digestion of the cyst wall by the juices of the host nor yet by the ciliate itself, as described in Colpoda by Goodey (1913). The rupture is evidently due to internal pressure and occurs at the mechanically weakest spot. The shape of the cyst, alone, makes the base of the operculum the most vulnerable point; and moreover, as has been shown, both layers of endocyst are thin in this region. The experiments with hypotonic solutions indicate that the internal pressure is not due to osmosis alone; the cyst does not rupture even in distilled water. Nor is it due to swelling of the organism by imbibition, as in *Lembus pusillus*. On the contrary the body shrinks and fluid accumulates round it. A possible explanation of the pressure is that it is due to the formation of water by the breaking down of the body proteins during katabolism. *N. ovalis* resembles *Lembus pusillus* and the parasitic amoebae so far observed, in emerging from the ruptured cyst wall entirely through the activity of its own locomotor organelles.

The subject of the mode of development of a highly organized Protozoon from its encysted stage seems one worth further investigation. In the case of *Nyctotherus ovalis* a striking feature is the apparent completeness of cytoplasmic dedifferentiation preceding it. The reversal of polarity indicates this, though it may possibly be achieved by substances from one end of the body actually flowing to the other during cyclosis. More probably, however, the cytoplasm becomes, as it appears, more or less homogeneous. There seems little doubt that the ciliary apparatus is completely reorganized. Hoare (1927) suggests that in *Lembus pusillus* it may possibly be merely "withdrawn," since the surface striations often do not disappear at all and cilia form at excystation with great rapidity. In *N. ovalis,* however, this can hardly be the case. Owing to the situation of the mouth, the arrangement of the ciliary rows at the two poles of the body is different (Fig. 5), and when the poles are reconstructed in reverse positions the cilia must arise with a new arrangement. This accounts for the disappearance of striations for a brief period at the beginning of excystation. Unlike that of *Lembus pusillus,* the mouth in the ripe cyst is entirely obliterated, and the fresh one arises, with its membranelles, in a new position.

The reorganization process, in *N. ovalis* at least, should be regarded as a true development, in the sense that it is progressive differentiation from almost, or completely, dedifferentiated protoplasm. A knowledge of the point of origin of the mouth and mode of development of membranelles etc., in other species, may possibly throw light on fundamental problems. It may even prove permissible to draw conclusions from the process, regarding the evolutionary origin of the organoids. The statement of Ilowaisky (1926) regarding the apparent development of the cirri from free cilia, in *Stylonichia mytilus*, is interesting in this connection.

EXPERIMENTS WITH THE CYSTS OF *Endamoeba blattae*

As stated above, a considerable amount of work was carried out with the object of finding excystation, and subsequent stages in the life history of *Endamoeba blattae* (Bütschli) Leidy. Since existing data (Mercier, 1910) were founded on the study of stained sections, efforts were directed towards finding the excysting organism alive. Accordingly the method used was that described above for excystation of *Nyctotherus ovalis in vivo*.

The main difficulty, as has been said, was the scarcity of cysts. This was also found by Elmassian (1909), Schubotz (1905) and by other workers, but not apparently by Mercier. He says that he was able to give to experimental insects "une nourriture largement et fréquemment saupoudrée de kystes." Cysts occurred even more sporadically than in *N. ovalis*. Concentration was impracticable without allowing feces to accumulate long enough for degeneration to set in.

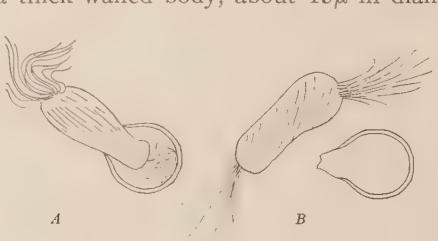
In spite of the scarcity cysts as they were available were fed to cockroaches on ninety different occasions. A remarkable fact revealed was the very high percentage of nonviable cysts. Schubotz (1905) after unsuccessful experiments concluded that probably only 10 to 20% were capable of development. The present work suggests that the figure may be even lower. Cysts were constantly met with in the midgut apparently entirely unaltered, even 24 hours after the feed. They were sometimes kept in the medium for another day without showing any change. Yet their appearance was often normal. Probably the time taken for the cyst to ripen is the factor entering in here. The mature form contains 40 to 50 nuclei, and cysts are liable to be passed out of the body before the full number of nuclear divisions have been accomplished. Accurate counts to determine maturity cannot, of course, be made in the fresh state. Other nonviable cysts underwent various degenerative changes. The only observed stage of development occurring in the ingested cysts was an early phase. This was not infrequently found. The central part of the protoplasm was granular, while the

remainder had become more hyaline; the numerous nuclei had all migrated to the peripheral region. This observation confirms Mercier's account of the preliminary phase; but no further development took place. Empty cyst walls were, however, occasionally found.

Exposure of *Endamoeba blattae* cysts to higher temperatures, by the method used for *N. ovalis,* had no effect at all.

EXCYSTATION IN *Lophomonas striata*

The cysts of *Lophomonas striata,* another parasite of the cockroach's hindgut, have lately been redescribed by Kudo (1926). According to his and other accounts of stained preparations, the ripe form has two nuclei and bears no traces of flagella or axial filaments. According to Kudo it retains the "ectoplasmic rods" of the trophozoite; but Grassé (1926), who interprets the rods as ectoparasitic Schizomycetes, states that they are often absent in the cyst. In the fresh state the cyst appears as a thick-walled body, about 15μ in diameter. Within it



TEXT FIGURE

Excystation of *Lophomonas striata* (living specimen). (A) Trophozoite emerging from the cyst with a crown of flagella at each end. (B) The same after emerging. The empty cyst wall is shown. × 625 approx.

nothing is visible except, in many cases at least, refractile streaks in the protoplasm, which represent the "rods" referred to. They were present in the organism described below.

In a single experiment excystation of this species was observed. An adult *B. orientalis* was fed feces in which there were cysts of *E. blattae,* and, as it happened, of *L. striata* also. Twenty-four hours later the contents of the alimentary canal were examined, and most of the meal found to have passed into the hindgut. In the latter organ an excysting *L. striata* was observed, and the cyst wall had already ruptured at one point. The organism within was elongated and had developed a crown of flagella at both ends (Textfig.). Part of it already protruded, and the flagella at the outer extremity appeared to be longer and more active than the others. In 5 minutes by the activity of the flagella the organism had extracted itself from the wall, and this was left intact except for the relatively small aperture. The flagellate was unfortunately lost about half an hour later. There is

little doubt however that the excysted body is double in nature and shortly undergoes transverse fission. It would thus agree with the genus Giardia except for the mode of division. Though the specimen described was found in the hindgut, this may not be the normal locality for excystation. Trophozoites, apparently freshly excysted, of both *Lophomonas striata* and *L. blattarum* have been found several times in the midgut. It is here that they probably emerge as a rule.

CULTURE WORK

The work of Barret (1928) in culturing *E. thomsoni* has been confirmed. This intestinal amoeba of the cockroach, described in an earlier paper (Lucas, 1927), has been grown on Barret's serum-saline medium, and on modifications of it. The same culture method also proved very favorable to the small flagellate *Monocercomonas orthopterorum* Parisi, emend. Bělař. This organism multiplied copiously and was able to survive without subinoculation for over a month. The medium has not, however, been successful with *N. ovalis* or *Endamoeba blattae*. The great majority of individuals of these species die in it almost at once, and those which survive appear to be living by feeding on the fecal debris present in the tubes. Slight changes in the salt content and the addition of starch by the method of Dobell and Laidlaw (1926) caused no improvement. Dividing forms of *N. ovalis* have been found but the species has not survived more than one subinoculation (19 days in all).

Endamoeba blattae has not lived more than 14 days, and dividing forms have never been seen. On one occasion after 11 days in the culture tube a few of these amoebae were placed in the medium in a depression slide and the coverslip sealed. Some of them lived thus for 6 days and were frequently examined. Most of them eventually formed a peculiar type of cyst which had sometimes been met with in the cultures. The numerous nuclear divisions which normally precede encystment did not occur. The amoebae became sluggish and the nuclear membrane, usually 1μ in thickness, became much thinner. A normal-looking, thick cyst wall was then secreted, and by the time it was formed the nucleus was no longer visible. It seems probable in such cases that the attenuated nuclear membrane ruptures. The fully formed cysts all appear entirely enucleate and are probably lifeless. Experiments to test their viability, however, cannot at present be conclusive owing to the negative results so far obtained with the normal type of cyst. Elmassian (1909) noticed a precipitation of encystment, preceded by thinning of the nuclear membrane, when a hindgut containing amoebae was left in physiological salt solution. The process seems to have resembled that described above. When it was rendered slower by exposing to a temperature of 12° C., several nuclear divisions

occurred within the wall. Hence he obtained what he termed "clear cysts" as opposed to "dark cysts" also to be found in the hindgut. But he interpreted them both as part of the normal life cycle of the amoeba. Owing to its absence in natural conditions, such cyst-formation should probably be regarded as a pathological reaction to unfavorable environment.

SUMMARY OF RESULTS

Nyctotherus ovalis in stages of excystation has been obtained from the stomach of cockroaches which were previously fed with the cysts. The cytoplasm of the cyst is almost entirely dedifferentiated when it is ripe, but the polarity of the cyst wall is well-marked.

All stages in the development of the ciliate from the encysted body have been followed continuously under the microscope, the process occupying about 6 to 8 hours. In the first phase cyclosis occurs. Dedifferentiation seems to be completed and the surface striations, if still present, disappear. The anterior end of the ciliate forms from cytoplasm in the posterior end of the cyst, so that polarity is reversed. The ciliary apparatus appears to be formed entirely anew. The contractile vacuole develops when the body begins to rotate within the cyst wall. The point of exit is predetermined and is covered by a definite operculum, situated at what was the posterior end of the encysted organism. The operculum is ultimately pushed off by internal pressure and the organism emerges by ciliary activity.

Experiment shows that intake of water from a hypotonic medium by endosmosis, if it occurs at all, cannot stimulate *N. ovalis* to encyst, and is insufficient to rupture the wall. Treatments with various digestive juices had little or no effect on *N. ovalis* cysts. The whole process of excystation has been induced in 0.5% sodium chloride solution by maintaining a temperature of 28 to 30°C.

Numerous attempts with *Endamoeba blattae* have failed to disclose the mode of excystation and subsequent life history. The cysts are extremely scarce and a high proportion nonviable.

Lophomonas striata has been found excysting in the gut of the cockroach. It emerges as a double organism.

The success of the culture medium described by Barret (1928) for *Entamoeba thomsoni* is confirmed. In this medium *Endamoeba blattae* did not survive, but formed abnormal cysts.

The writer wishes to express her gratitude to Dr. Robert Hegner for his suggestions and advice connected with the work; also to acknowledge her indebtedness to the University of London for a Studentship which enabled her to undertake the research, and to the Johns Hopkins University for the facilities given in the laboratories of the School of Hygiene and Public Health.

References

Abbot, R. 1926.—Contributions to the physiology of digestion in the Australian roach *Periplaneta australasiae* Fab. Jour. Exp. Zool., 44:219.

Barret, H. 1928.—(Paper in press.)

Bodine, J. 1923.—Excystation of *Colpoda cucullus*. Jour. Exp. Zool., 37:115.

Cutler, D. 1919.—Observations on *Entamoeba histolytica*. Parasit., 11:127.

Dobell, C. and Laidlaw, P. 1926.—On the cultivation of *Entamoeba histolytica* and some other entozoic amoebae. Parasit., 18:283.

Elmassian, M. 1909.—Sur *l'Amoeba blattae*. Arch. f. Protisenk., 16:143.

Goodey, T. 1913.—The excystation of *Colpoda cucullus* from its resting cysts, and the nature and properties of the cyst membranes. Proc. Roy. Soc., B 86:427.

Grassé, P. 1926.—Contribution a l'étude des Flagellés parasites. Arch. zool. exp., 65:345.

Hegner, R. 1927.—Excystation in vitro of human intestinal Protozoa. Science, 65:577.

Hoare, C. 1927.—Studies on coprozoic ciliates. Parasit., 19:154.

Howaisky, S. 1926.—Material zum Studium der Cysten der Hypotrichen. Arch. Protistenk., 54:92.

Kudo, R. 1926.—A cytological study of *Lophomonas striata* Bütschli. Arch. f. Protistenk., 55:504.

Lucas, C. 1927.—Two new species of amoeba found in cockroaches: with notes on the cysts of *Nyctotherus ovalis* Leidy. Parasit., 19:223.

Mercier, L. 1910.—Contribution a l'étude de l'amibe de la Blatte (*Entamoeba blattae* Bütschli.) Arch. Protistenk., 20:143.

Sanford, E. 1918.—Experiments on the physiology of digestion in the Blattidae. Jour. Exp. Zool., 25:355.

Schubotz, H. 1905.—Beiträge zur Kentniss der *Amoeba blattae* Bütschli und *Amoeba proteus* Pall. Arch. f. Protistenk., 6:1.

Smith, S. 1927.—Excystation in *Iodamoeba williamsi* in vivo and in vitro. Science, 65:69.

Yorke, W. and Adams, A. 1926.—Observations on *Entamoeba histolytica*. I. Development of cysts, excystation and development of excysted amoebae in vitro. Ann. Trop. Med. and Parasit., 20:279.

Explanation of Plate VI

All figures are drawn from living specimens of *Nyctotherus ovalis* Leidy, obtained from the stomach of experimentally infected hosts. All drawings were made with camera lucida except Fig. 4. In Figs. 5-7, however, where the organism represented was in rapid motion, most of the detail was inserted freehand. Magnification × 750 approximately.

Fig. 1.—Ripe cyst containing spindle-shaped reserve food bodies. The position of the nucleus indicates the anterior end of the encysted organism.

Fig. 2.—The same in an early stage of excystation. Cyclosis is occurring and the nucleus has moved towards the operculum end of the cyst, so that polarity is reversed. A clear area indicates the future position of the peristome and cytopharynx.

Figs. 3-7.—Stages in the excystation of a single individual. Spindle-shaped bodies were absent in this cyst.

Fig. 3.—Dorso-lateral view. A stage a little later than that in Fig. 2, showing striations representing the bases of the future membranelles. Surface striations have disappeared. The small food granules are aggregating at the anterior end.

Fig. 4.—A composite drawing to show the appearance of the stage in Fig. 3 if viewed from the dorsal surface. The grooved peristomial area is seen on the right.

Fig. 5.—Surface view of the right side of a later stage, showing the arrangement of the striae (the ciliary grooves). Those of the ventral surface are continuous with the membranelle-bases of the peristomial area, the more heavily striated region. The small cytostome lies at the posterior end of the peristome. Surface cilia and the contractile vacuole have developed.

Fig. 6.—Dorsal view of the ciliate emerging from the cyst. The operculum is lying free. The membranelles are now distinct. (For the sake of clearness, surface striae are omitted in Figs. 6 and 7).

Fig. 7.—Ventral view of the newly excysted ciliate. The posterior of the two transverse "septa" is beginning to differentiate.

PLATE VI

HAPTOPHRYA MICHIGANENSIS SP. NOV.[1]

A PROTOZOAN PARASITE OF THE FOUR-TOED SALAMANDER

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Haptophrya michiganensis, a new species of astomatous Protozoa, was discovered in the digestive tract of the four-toed salamander, *Hemidactylium scutatum* (Schlegel), by Dr. F. N. Blanchard who examined several thousand of these salamanders from Washtenaw and Livingston Counties, Michigan, and found about 70% of them infected with this protozoan (Blanchard, 1925). He later found one specimen of *Ambystoma jeffersonianum* from Livingston County which was also heavily infected. Through the kindness of Dr. Blanchard, I was given the opportunity to study this species. The parasites were secured in abundance from the anterior part of the intestine of the host. Some parasites were found in the rectum, especially when infection was heavy. These specimens, if free amid the débris, were nearly always of the small "bud" type. If in cases surrounded by a mucous coat, they were long or in any stage of division.

In order to secure parasites for the preparation of *in toto* mounts, the digestive tract of the host was removed, opened from end to end and placed in a watch glass with tap-water. If parasites were present they soon left their place of attachment and moved freely in the water. After removal of the intestine and fecal materials, Bouin's fluid was poured into the dish without removal of excess water. The parasites were then removed to small shell vials. Alcohols and stains were changed by means of a small pipette without injury to the specimens. For sections of the parasite the intestine of the salamander with the parasites in situ was fixed in Bouin's solution, dehydrated, cleared and embedded in paraffin. Longitudinal sections were made and stained with Heidenhain's iron hematoxylin. Some sections were counterstained with eosin, others with eosin and light-green.

The parasite is a ciliated organism, visible to the unaided eye and appearing like a small rhabdocoele worm. Ten unfixed single-celled specimens varied in length between 1.132 and 1.580 mm. Four features stand out: (1) a cup-shaped sucker in the anterior portion, facing ventrad; (2) the long nucleus; (3) the long contractile vacuole, straight or convoluted, beginning dorsad to the sucker and extending to the posterior end; (4) in newly budded specimens, two very short tips may be seen on the lateral margins of the posterior end.

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1. Contribution from the Department of Zoology, University of Michigan.

The entire body is covered by a thin pellicle which thickens in the anterior ventral region into the depression designated as the sucker. The pellicle is quite permeable because in 0.7% salt solution plasmolysis takes place very quickly and the animal appears to have a "neck," due to shrinkage in the anterior vacuolated region. The pellicle, however, offers resistance to pressure and mechanical change. The entire surface of the body, including the sucker, is covered with a nearly uniform, thick covering of fine cilia, arranged in longitudinal rows running in a spiral, turning to the right, when viewed from in front. The cilia of the body average 3.8μ in length. In the sucker they attain a length of 6.5μ.

The cytoplasm is divided into two distinct regions, ectoplasm and endoplasm. The ectoplasmic layer averages 2μ in thickness but attains its greatest thickness in the basal part of the sucker, where it reaches a thickness of 4.8μ. The ectoplasmic layer shows very definite alternating light and dark striations which are best seen in cross-sections (Fig. 2). There is a definite plane of demarcation between ectoplasm and endoplasm, closely resembling that described by Sharp (1914), for Diplodinium. When the parasite is unattached its sucker is cup-shaped and shallow, but when attached to the host the sucker is deeply hollowed out. The sucker serves for attachment to the intestinal wall of the host, as many as twelve epithelial cells being drawn into the cavity. No spines or hooks could be found in any of the suckers examined. Careful study of cross-sections failed to reveal an opening through the sucker into the body, and abscence of solid débris within the body of the parasite is also indicative of the lack of a mouth opening. When placed in a carmine suspension living parasites failed to ingest any of the carmine. The endoplasm is divided into two layers. The layer adjacent to the ectoplasm is uniformly granular in appearance; the inner layer highly vacuolated. In the anterior region next to the sucker and around the nucleus it forms a thick layer but posterior to the nucleus it is constricted to a narrow core.

This protozoan is bi-nucleated. The macronucleus (Fig. 1) is large, varying in fixed specimens from 0.105 to 0.178 mm. in length, about one-fifth the length of the body. In diameter it varies between 0.02 and 0.038 mm. In the "resting" stage it is to be seen in the anterior third of the body, posterior to the sucker. In fission the macronucleus migrates to the center of the cell and undergoes a division similar to that occurring in the macronucleus of Paramoecium. The macronucleus is surrounded by a reticular "basket" which is clearly brought out in sections stained with light-green. A peculiar feature of the macronucleus is its segmented appearance (Fig. 3). Scattered through the macronucleus are numerous chromatin granules varying in size up to 3μ, many of them showing stages of division (Fig. 2). These granules stain

heavily with Heidenhain's hematoxylin and are only revealed when the destaining is allowed to proceed for a long time.

The micronucleus which stains only with eosin lies in a pocket of the macronucleus and is covered by a distinct membrane. Division stages of the micronucleus were found when the macronucleus was yet undivided, indicating that division of the micronucleus precedes division of the macronucleus. Each half of the micronucleus migrates to the ends of the macronucleus and apparently divides several times, for as many as five micronuclei were seen in a single cross-section. The distribution of micronuclei on the macronucleus is shown in figure 6 drawn from a longitudinal section. It seems probable that these micronuclei are distributed to the buds at the time of division.

Cohn (1904) refers to the large body seen in the four-bud stage of a related species, *Haptophyra gigantea,* as "the micronucleus making its appearance." In many sections of *H. michiganensis* a similar body can be seen to arise as the result of a pinching-off process of the extremity of the macronucleus distal to the most recent fission plane (Figs. 5 and 7). The final stage of this body pinched off from the macronucleus is seen in a mass of small granules, indicating that this is a disintegration process by means of which the size of the macronucleus is reduced. This body is therefore not to be confused with the micronucleus which is a distinct structure plainly seen in all stages and positions of the macronucleus. This body described by Cohn and the macronucleus stain heavily with hematoxylin showing them to have the same chemical structure. It will be recalled that the micronucleus takes only the eosin stain.

The contractile vacuole is a long thick-walled tube beginning in the anterior end, dorsal to the sucker and extending almost to the posterior end where it is attached to the dorsal surface. The tube lies between the ectoplasm and endoplasm, opening at regular intervals by short tubes through the pellicle, forming a linear series of seven to nine excretory pores (Fig.1). Maupas (1879) describes this type of canal in *H. gigantea* as having seven to nine pore openings for the discharge of liquid wastes. Certes (1879) in describing the same species said he was unable to find the pores. In my specimens, when India ink was used as a medium, the explosion of the vacuole through the pores could be seen. The explosion begins at the anterior pore and progresses rapidly down the series to the posterior pore where the largest explosion takes place. This pore is located about one-eighth of the body length from the posterior end indicating that the canal ends in a blind sac. In the taxonomy of the ciliates, the contractile vacuole is considered to be of great importance. In the ciliated Protozoa the contractile vacuole shows an evolutionary development from a single vacuole through a linear series of vacuoles,

to a "canal" having a series of openings. The species of Haptophrya stand at the end of this evolutionary series.

Asexual reproduction by linear budding is the common method of multiplication. The method as described by Maupas (1879) and Cépède (1910) for *H. gigantea* is followed very closely by *H. michiganensis*. Examples of specimens showing two cells in a chain are abundant, but specimens with three to six buds are rare. Six buds is the largest number observed in the present species, but Cépède (1910) has illustrated specimens of *H. gigantea* showing seven and eight buds in a chain. It is my belief that the formation of long chains may be of seasonal occurrence or perhaps due to some condition of the host. On removal of the parasites from the salamander, the buds separate easily, thus preventing observation of long chains in *H. michiganensis*.

Cépède (1910) described a related species, *Anoplophrya maupasi*, which undergoes a conjugation cycle similar to that of Paramoecium and also an encystment stage which he claimed to be typical of Haptophrya. He did not, however, give any evidence to support his claim. Neither conjugation nor encystment have been recorded by earlier investigators for Haptophrya and all my attempts to discover these phenomena in the present species have failed. In an attempt to discover the sexual stages, parasites were cultured in various media. In some of these media the parasites were able to maintain themselves for a period of ten days. In a later experiment parasites were carefully washed in several changes of half strength physiological salt solution, and in this solution they remained alive for thirty days. Bacteria were kept at minimum numbers by keeping the temperature low. The parasites showed no indication of multiplication during the course of the experiment.

The genus Haptophrya with *H. gigantea* as the type species was created by Stein to include those astomatous, ciliated parasites without a neck-like constriction, which are found in the digestive tract of batrachians. The genus Discophrya Stein* (type species—*D. planariarum*), was set up to include the astomatous parasites with a neck-like constriction which inhabit turbellarians and other invertebrate hosts. Both genera belong to the family Opalinidae. The present species, having no neck, clearly falls in the genus Haptophrya.

For the purpose of comparison with material of the type species, two specimens of *Discoglossus pictus* were secured from Algeria, North Africa from which region *H. gigantea* has been reported. One host yielded but ten and the other about one hundred specimens of

* Calkins (1926) records a genus Discophrya in the class Suctoria, as well as a genus Discophrya, class Ciliata. This duplication is also followed by Wenyon (1926). Dr. Calkins, in a letter of January, 1928, states in regard to this double use that the genus Discophrya, family Opalinidae, class Ciliata, has priority and should be retained.

H. gigantea. These specimens agreed with the length of 1.260 to 1.600 mm. and the description given by Cépède (1910) for *H. gigantea* found in the batrachia from northern Africa. The length of these specimens is slightly greater than the length of *H. michiganensis,* which averages 1.286 mm. in 10 unfixed specimens. *H. michiganensis* is less opaque than *H. gigantea* and thus the nucleus in the former is more conspicuous. A very noticeable difference exists in the number and length of body cilia. *H. gigantea* has a most striking covering of long cilia, while *H. michiganensis* has a greater number of cilia but the cilia are neither so long nor as conspicuous. The sucker of *H. michiganensis* is better defined and more nearly circular than that of *H. gigantea.* The sucker of the latter is larger, but its margin is not so clearly defined anteriorly. This large sucker produces a flaring out of the anterior end of the parasite which is not noticeable in *H. michiganensis.* When the two species are placed in the same dish *H. gigantea* attaches itself to the bottom readily and very firmly, while specimens of *H. michiganensis* move about freely, rarely attaching themselves, and when they do fasten themselves, the attachment is insecure. The diameter of the cell in *H. michiganensis* does not vary appreciably in the course of its length, while *H. gigantea* tapers slightly from anterior to posterior terminating in a more pointed end than that of *H. michiganensis,* whose posterior ends tapers sharply to a rather blunt ending.

The differences noted between the Afro-European and American forms of Haptophrya are believed to be significant and for the American form a new species *Haptophrya michiganensis* is created.

Paratypes of *H. michiganensis* have been placed in the Museum of Zoölogy of the University of Michigan, Division of Parasitology, where the lot number is 199.

SUMMARY OF RESULTS

1. *Haptophrya michiganensis,* an astomatous protozoan parasite, has been found in quantity in the intestine of the four-toed salamander, *Hemidactylium scutatum,* and in a single specimen of *Ambystoma jeffersonianum.*

2. It is possible to keep the parasite alive in half normal physiological salt solution for thirty days.

3. A comparison with *H. gigantea* shows the Michigan species to be new.

References Cited

Blanchard, Frank N. 1923.—The Life History of the Four-toed Salamander. Amer. Nat., 57:262-268.

Calkins, G. N. 1926.—The Biology of the Protozoa. 623 pp., New York.

Cépède, C. 1910.—Recherches sur les Infusoires astomes. Anatomie, Biologie, Ethologie parasitaire, Systematique. Arch. zool. exp., (Ser. 5) 3:341-609, 47 text-figs., 9 pls.

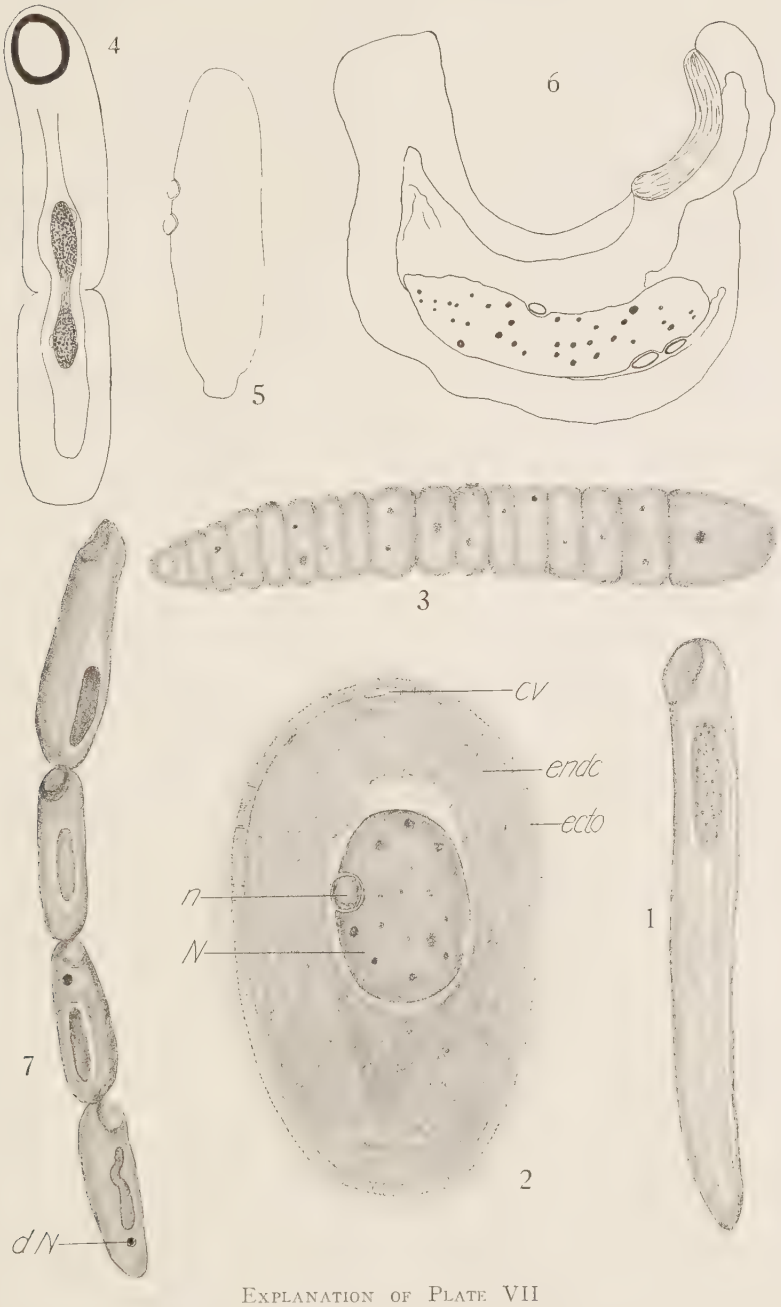
Certes, A. 1879.—Note sur *l'Haptophrya gigantea* Maupas infusoire parasite des batraciens anoures d'Algérie. Bull. Soc. Zool. France, 4:240-244.

Cohn, Ludwig. 1904.—Zwei parasitische Infusorien aus *Discoglossus pictus*. Arch. Protistenk., 4:43-63, 1 pl.

Maupas, E. 1879.—Sur *l'Haptophrya gigantea* opaline nouvelle de l'intestin des batraciens anoures d'Algérie. Compt. rend. Acad. sci. Paris, 88:921-923.

Sharp, R. G. 1914.—*Diplodinium ecaudatum* with an account of its neuromotor apparatus. Univ. Calif. Pub. Zool., 13:43-122, 7 pls.

Wenyon, C. M. 1926.—Protozoology. 2 vols., 1563 pp., London.



EXPLANATION OF PLATE VII

Fig. 1.—Drawing of a whole specimen in side view.

Fig. 2.—Cross-section showing micronucleus.

Fig. 3.—Longitudinal section of the macronucleus showing structure.

Fig. 4.—Single individual dividing.

Fig. 5.—Macronucleus showing micronucleus dividing and pinching off of part of macronucleus.

Fig. 6.—Longitudinal section of individual showing macronucleus and three micronuclei.

Fig. 7.—Chain of 4 individuals.

GLAPHYROSTOMUM SANGUINOLENTUM A NEW TREMATODE *

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A search of the literature shows that the genus Glaphyrostomum Braun of the family Harmostomidae is represented only by the two species *G. adhaerens* Braun 1901 and *G. propinquum* Braun 1901. Therefore, the trematode presented in this paper, *G. sanguinolentum,* is probably the third species of the genus to be described. It is of further importance since, like *Ityogonimus talpae* (Goeze 1782) Witenberg, it represents a parasite which causes pathological changes in the body of its host; these two species are apparently the only two Harmostomidae which have been found to have injurious effects. *I. talpae* has erroneously been described as *Ityogonimus lorum* (Dujardin) Lühe by several authors (Melnikow 1865:44-55; Looss 1899:652; and Gonder 1910: 169-170). This mistake was corrected by Witenberg (1925:179-180), and the priority right of the name *I. talpae* re-established. In the same paper, Witenberg (1925:178), drew attention to the priority of Lühe in the naming of the genus Ityogonimus Lühe 1899. Gonder (1910:169-170) was the only one of the authors to mention the fatal effects of *I. talpae* on its host.

Three mature specimens of *G. sanguinolentum* were found in the small intestine of a MacGillivray warbler (*Oporornis tolmiei* Towns) near Friday Harbor, Washington. When found, the parasites were opaque and deeply red with blood, showing their pathogenicity. It is not likely that death was caused by the parasitism, as evidently was the case with *I. talpae* (Gonder, 1910:170).

Superficially, *G. sanguinolentum* might be classified as a Harmostomum, so characteristic is its appearance of the family Harmostomidae. Two features, however, are quite distinctive; the anterior end of the body is rather elongated, giving the appearance of a slender neck following the enlarged region surrounding the oral sucker (Fig. 1). In common with *I. talpae* (Gonder 1910:171) and *I. lorum* (Dujardin 1845:407), *G. sanguinolentum* shows the prominent papilla on which the genital pore opens. This is so enlarged that it projects from the ventral surface, being especially striking from a lateral view (Fig. 2). Relationship

* The preparation of this paper was started at the Puget Sound Biological Station, Friday Harbor, Washington, and completed at the Department of Zoology, University of Washington. At this time I wish to express my gratitude to Dr. John E. Guberlet, of the Department of Zoology, University of Washington, for assistance and criticism, and to Dr. Maria Heinemann of Bellingham, and Mrs. Frida Werby, of Seattle, Washington, for translations of references.

with the genus Glaphyrostomum is established by the position of the genital pore situated posterior to the anterior testis, the two strongly developed suckers, and the proximity of the genital glands to each other.

The body of the worm is long, cylindrical, or tongue-shaped, and flattened on the ventral surface. It measures 2.32 mm. in length, its width varies from 0.248 mm., just past the oral sucker, to 0.53 mm., about three fifths of the body length from the anterior end. From this point the body tapers gradually anteriorly; posteriorly it diminishes more suddenly. Very likely a specimen not so filled with eggs would show a more uniform width.

No spines are present, the cuticula being perfectly smooth. Both the anterior and ventral suckers are well developed, the former a little more so; the anterior measures 0.274 by 0.241 mm., it is deeply cup-shaped, opening forward; the transverse mouth is 0.116 by 0.074 mm. Deep-set, yet with the lips projecting slightly from the ventral surface, the circular ventral sucker measures 0.252 by 0.248 mm. It is located about one third of the body length from the anterior end, at about the region where the body begins to widen. Following the oral sucker is the well developed pharynx, 0.130 by 0.139 mm.; this is presumably followed by a very short esophagus, which is almost indistinguishable, its origin being encircled by the pharynx, and the remainder by the intestinal crura. Apparently surrounding the posterior part of the esophagus, the intestinal diverticula extend laterally, then making a right-angled bend posteriorly, pass, in an almost straight course, to the posterior end of the animal, ending in ceca which are close together.

Ventral to the digestive tract are the organs of reproduction, which occupy practically the entire width of the body, from a point a short distant posterior to the intestinal crura to a point almost within the posterior ends of the intestinal ceca. Great similarity is here evident to the arrangement of these glands in Harmostomum. Here again the reproductive organs occupy the posterior end of the body, the two testes lying tandem with the ovary between them; the three glands are very close together, well near the caudal end of the animal. The posterior testis, flattened dorso-ventrally, measuring 0.117 by 0.104 mm., lies in the mid-line; the anterior, flattened laterally, is somewhat to the left of the mid-line; it measures 0.126 by 0.091 mm. (fig. 3). From the mid-ventral portion of the posterior testis, its vas efferens extends forward and to the left, meeting the vas efferens of the anterior testis ventrally; the vas deferens formed by their union enters the much lobulated seminal vesicle which lies in the mid-line, anterior to the genital glands and dorsal to the posterior portion of the uterus. Lying free in the parenchyma is the almost straight ductus ejaculatorius, which continues into the rather muscular-walled cirrus pouch as a slightly coiled penis. The pars prostatica was not visible. Between and dorsal to the two

testes, and a little to the right, is the somewhat larger ovary, measuring 0.165 by 0.109 mm. Lateral and caudal to the posterior portion of the ovary is the ootype, while between the two is the shell gland. There is no seminal receptacle, but Laurer's canal is present, emptying dorsally at the level of the anterior portion of the ootype. Beginning about the level of the middle of the ovary, the uterus, filled with eggs, occupies the whole ventral portion of the body, even underlying the intestinal ceca, to a point almost as far anterior as the origin of the intestine; here it is not so large. Bending on itself, the uterus descends to open from the right side into the vagina. This organ, although the tube is larger, is not so thick-walled as the penis. The vagina is more coiled, descending alongside the cirrus pouch, and emptying through the genital pore from the left. On account of the prominent genital papilla, the genital pore is very easily seen; it opens on a level with a point just posterior to the middle of the ovary. Clearly visible, even in the living animal, are the vitelline glands which arise, as follicular units, near the anterior border of the ventral sucker, extending to a point near the posterior margin of the ovary. Passing almost directly medially, and ventral to the reproductive glands, the two slender vitelline ducts meet in the center as an oval vitelline receptacle. Gently pulsating, these ducts and reservoir were among the most prominent features of the living animal. Yellowish brown eggs filled the entire uterus, giving their color to the body washed free of blood, but showing up darkly in the parasite as just removed from the host, and stained with blood. These eggs are oval, rather saucer-shaped discs, measuring 21 to 23 by 10 to 14μ. It was impossible to see the excretory system, even in the living animal. An excretory vesicle could be discerned, in the mid-line, just caudad to the posterior testis; slightly behind this, but not at the extreme caudal tip, the excretory pore opens.

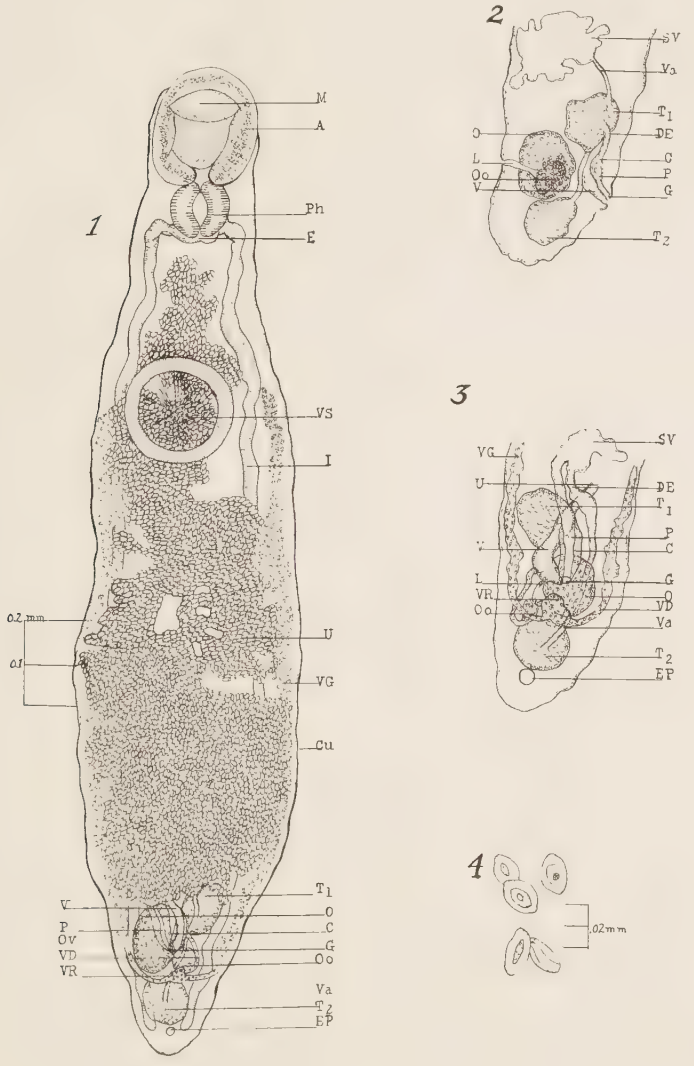
G. sanguinolentum corresponds more closely to *G. propinquum* Braun than it does to *G. adhaerens* Braun. In size, *G. propinquum* is slightly larger (2.7 by 0.7 mm.); both are cylindrical and both are free of spines, in contrast to *G. adhaerens*, which is spined at the anterior end. A difference is noted in the development of the suckers; whereas in *G. adhaerens* and *G. propinquum* the ventral sucker is larger than the anterior sucker in *G. sanguinolentum* the anterior sucker is slightly the larger. It shows the transverse mouth opening characteristic of the genus, but in common with *G. adhaerens*, does not show any of the local thickenings of the oral sucker so prominent on *G. propinquum*. While the ventral sucker of *G. adhaerens* is close to, or in the middle of the body, in the other two species the sucker lies one third of the body length from the anterior end. All three forms show a strong pharynx, in every case broader than long; *G. propinquum* only has a short, contracted prepharynx, and *G. sanguinolentum* alone shows a small

imbedded esophagus. Judging from the drawings and descriptions by Braun (1902:131-132, figs. 78-79), *G. adhaerens* and *G. propinquum* both show the loops of the intestine running anteriorly just following the diverticulum, which arrangement is characteristic of the genus Harmostomum; *G. sanguinolentum* does not show this peculiarity. According to the drawings by Braun (1902, figs. 78-79) a few of the posterior lateral loops of the uterus lie ventral to the intestinal ceca; in the description of *G. propinquum,* Braun (1902:133) definitely mentions this point of structure; in *G. sanguinolentum* the uterine loops lie ventral to the ceca and even to portions of the vitelline glands. In all three forms the ovary is larger than the testes; all lack a seminal receptacle, but have Laurer's canal. There is a slight difference in the position of the genital pores: *G. adhaerens* has the genital pore between the ovary and the posterior testis; *G. propinquum* and *G. sanguinolentum* have the genital pore at the level of the posterior half of the ovary. The ductus ejaculatorius of *G. adhaerens* has a thick circular muscle, this is not evident in the other two forms. A striking differentiation is shown in the extent and arrangement of the vitelline glands: those of *G. adhaerens* extend from slightly behind the pharynx to the ovary, and are irregularly follicular; the vitelline glands of *G. propinquum* begin at the level of the pharynx, and extend to the ovary, the follicles here, however, lie in distinctly transverse clusters. *G. sanguinolentum* has follicular vitelline glands which extend from the level of the ventral sucker to the ovary; peculiarly, in all three forms the vitelline glands do not start at the same level on both sides. The eggs of *G. sanguinolentum* are comparatively larger, 23 by 17μ, as against 20 by 9 to 10μ for *G. adhaerens* and 28 by 11μ for *G. propinquum.*

The generic description given by Braun (1901:942) and modified by Witenberg (1925:221) is as follows:

Body tongue-shaped, mouth opening round or stretched transversely; turning point of uterus lies in front of ventral sucker; posterior ends of vitelline glands lie in a plane with the anterior part of the anterior testis; genital pore behind anterior testis.

G. sanguinolentum fits nicely into this generic description, except for the primary criterion for separation into tribes, the ending of the vitelline glands at the anterior part of the anterior testis, the criterion laid down by Witenberg (1925:177); curiously enough, all three species of the genus Glaphyrostomum, as stated above, have their vitelline glands ending on a level with the ovary. To correct this contradiction, I would suggest that the separation into tribes be based on the same point that Looss (1899:655) used in his separation of the family into the subfamilies Urogoniminae and Harmostominae, i. e., the terminal position of the genital pore for the genera Leucochloridium Carus 1835 (Urog-



EXPLANATION OF PLATE VIII

A, Anterior sucker; *C*, Cirrus pouch; *Cu*, Cuticula; *DE*, Ductus ejaculatorius; *E*, Esophagus; *EP*, Excretory pore; *EV*, Excretory vesicle; *G*, Genital pore; *I*, Intestine; *L*, Laurer's canal; *M*, Mouth; *O*, Ovary; *Oo*, Ootype; *Ov*, Oviduct; *P*, Penis; *Ph*, Pharynx; *SG*, Shell gland; *SV*, Seminal vesicle; *T*, Testis; *U*, Uterus; *V*, Vagina; *VD*, Vitelline duct; *VE*, Vas efferens; *VG*, Vitelline gland; *VR*, Vitelline receptacle; *VS*, Ventral sucker.

Fig. 1.—*Glaphyrostomum sanguinolentum*, ventral view.

Fig. 2.—Reproductive system, right sagittal view.

Fig. 3.—Reproductive system, frontal section, dorsal view.

Fig. 4.—Eggs of *G. sanguinolentum*.

onimus Monticelli 1888) and Urotocus Looss 1899, and a more anterior position of the genital pore in the genera Ityogonimus Lühe 1899, and Harmostomum Braun 1899.

On the basis of the above comparisons and discussion I would propose the following generic description:

Body tongue-shaped, cuticula smooth or spined. Anterior sucker round, oral opening transverse. Ventral sucker one third to one half the body length from the anterior end, circular. Powerful pharynx, broader than long, prepharynx and esophagus present or absent; intestine extends laterally and anteriorly from the diverticulum, then turning, runs to the posterior end of the body, the ceca lying close together in the caudal end. Ovary larger than testes; seminal receptacle lacking, shell gland and Laurer's canal present. Genital pore behind the anterior testis. Turning point of the uterus in front of ventral sucker, lateral uterine loops more or less ventral to intestinal ceca. Vitelline glands, beginning from pharynx to ventral sucker, extend to ovary. Eggs 20 to 23 by 9 to 17μ.

Bibliography

Braun, M. 1901.—Zur Revision der Trematoden der Vögel, II. Centralbl. Bakter. und Parasit., 29:941-948.

1902.—Fascioliden der Vögel Zool. Jahrb., Syst., 16:1-162.

Dujardin, F. 1845.—Histoire nat. des helminthes ou vers intestinaux, Paris.

Gonder, Richard. 1910.—*Ityogonimus lorum* (Dujardin). Centralbl. Bakter., I. Orig., 53:169-174.

Looss, A. 1899.—Weitere Beiträge zur Kenntniss der Trematoden-Fauna Aegyptens. Zool. Jahrb., 12:521-784.

Melnikow, Nicolaus. 1865.—Ueber *Distomum lorum* Duj. Arch. für Naturg., 31:49-55.

Witenberg, G. 1925.—Trematodenunterfamilie Harmostominae Braun. Zool. Jahrb., Syst., 51:167-254.

OBSERVATIONS AND EXPERIMENTS ON THE HAEMOGREGARINES OF CERTAIN AMPHIBIA *

ELIZABETH P. SANDERS

As a preliminary to a study of host-parasite specificity of the haemogregarines of frogs, a limited survey of these parasites found in the amphibia available at Cold Spring Harbor was made during the months of July and August, 1926. Practically all the animals examined came from one small pond, the Fourth Lake. As the animals were kept alive in the laboratory that the course of infection might be watched, the number of hosts examined was not large. Tadpoles, salamanders, toads and frogs were examined but frogs being most readily obtained were studied in particular.

As soon as the animals were brought into the laboratory blood smears were made, and the stained preparations examined for parasites. To get a quantity of blood sufficient to make a satisfactory smear, it was found best to bleed the frogs from the corner of the mouth. A small cut with fine scissors at this point yielded a good flow of blood, producing a wound that healed quickly. This operation could be repeated often without seriously injuring the frog. Tadpoles and salamanders were bled by snipping off a small piece of the tail. All smears were stained with Romanowsky stains (Wright or Giemsa). The frogs were then put into separate glass containers with wire gauze tops, thus protecting the animals against possible insect vectors of parasites. With daily changes of the small amount of water in which the frogs sat, and weekly feedings, it proved possible to keep them in isolated captivity. Tadpoles were kept in a similar manner, but no effort was made to keep toads and salamanders. They were liberated after an initial examination.

Records were kept of the haemogregarine type of parasite only. Two genera were observed, Karyolysus and Lankesterella. The parasites could be easily distinguished by size alone, Lankesterella being smaller, about half the size of the red blood cell, and Karyolysus about the same length as the cell. Morphological details also distinguished the two parasites.

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* This work was made possible by the grant of a research table by the Biological Laboratory, Cold Spring Harbor, Long Island, N. Y., and was greatly aided by the advice and assistance of Dr. Justin Andrews.

Haemogregarina has also been reported from frogs, but it was not observed in this survey. It was at first thought that mixed and pure infections of Karyolysus and Haemogregarina were found in some of the frogs, but this was later found not to be the case. According to Wenyon, Labbé, in 1894 when describing the parasite which he called Karyolysus took as one of the criteria for distinguishing it from Haemogregarina the karyolytic action it had on the nucleus of the cell. Reichenow has found that the life-cycle of Karyolysus in lizards differs from that of other haemogregarines, and that it therefore should be placed in a separate genus, but that the karyolytic action cannot be regarded as of generic value. The life-cycle of Karyolysus in the frog has not been completely worked out, but it seems probable that it is similar to that in the lizard. The only stages ever seen in the red blood corpuscles appeared to be gametocytes. Schizogony was never observed in the peripheral blood. In the genus Haemogregarina schizogony takes place in the red-blood cells, and if the parasites which had no effect upon the nucleus of the cell had been Haemogregarina, it seems certain that schizogonous forms would have been seen. For these reasons it was concluded that Karyolysus and not Haemogregarina was observed.

The karyolytic action of the parasite on the nuclei of the cells is a point of some interest though not of generic value. In the blood of some frogs, all of the parasitized cells had their nuclei fragmented, some had none fragmented, while others showed both fragmented and unfragmented nuclei. The degree of karyolytic action seemed to vary, and to be constant for the particular strain of parasite in the frog. Sometimes the nucleus was whole, sometimes broken into two or three pieces and sometimes fragmented into a greater number of small pieces, the type of shattering being constant for the particular frog. No doubt the parasites all belong to one species, but it appears that there may be different strains within the species, the particular strain being indicated by its karyolytic action. Frogs which showed both fragmented and unfragmented nuclei might be considered as having an infection with mixed strains. The cause and significance of this karyolytic action is not known.

Results of the initial examinations of the animals are presented in the accompanying table.

The points of interest indicated here are several. The adult green frogs, *R. clamitans,* were parasitized with Karyolysus and Lankesterella, but the young green frogs were parasitized with Lankesterella only, Karyolysus never being present. These young frogs were all brought into the laboratory as tadpoles, metamorphosing in isolated captivity. Any infection present must have been acquired before capture while in the tadpole stage.

The young bull frogs showed a heavy infection with Lankesterella, but Karyolysus was never observed. These frogs also metamorphosed in the laboratory as did the green ones. Lankesterella is transmitted from one animal to another by a leech. That these young frogs were infected with one parasite of known origin and not the other would seem to indicate that Karyolysus was not transmitted by a leech, but by a vector which could attack a frog, and not a tadople.

The tadpoles examined were of varying ages and species, but they nevertheless substantiated the findings in the known species of laboratory raised frogs, that is, they were never infected with Karyolysus, but were parasitized with Lankesterella. It was difficult to bleed the tad-

	No. of Animals	Parasitized		Unparasitized		Karyolysus		Lankesterella		Karyolysus and Lankesterella		Total Karyolysus		Total Lankesterella	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
R. clamitans Adult.....	67	57	85	10	15	29	43	9	13	19	28	48	71	28	41
R. clamitans Young.....	19	8	42	11	58	..	..	8	42	..	..	..	..	..	..
R. catesbiana Adult.....	2	1	50	1	50	1	..	..	..	..	..	..	..	..	..
R. catesbiana Young.....	20	12	60	8	40	..	..	12	60	..	..	..	..	12	60
R. palustris Adult.....	32	..	..	32	100	..	..	..	.	..	..	..	..	..	..
Young frogs species undetermined.	8	3	38	5	62	..	..	3	38	..	..	..	..	3	38
Tadpoles.....	37	4	11	33	89	..	..	4	11	..	..	..	..	4	11
Toads.....	3	..	..	3	100	..	..	..	..	..	..	..	..	..	..
Salamanders.	7	..	..	7	100	..	..	..	..	..	..	..	..	..	..
Young frog total.....	47	23	49	24	51	..	..	23	51	..	..	..	..	23	51

poles, with the result that the smears were often poor. This may account for the low incidence of infection among them.

The number of adult bull frogs, *R. catesbiana,* examined was too small to be significant, only two having been examined. They showed, however, that the species is susceptible to infection with Karyolysus. None of the pickerel frogs, *R. palustris,* ever showed either parasite. It would be interesting to know whether this was due to the habits of the frog, the transmitting agents for Karyolysus and Lankesterella, or a host-parasite specificity.

EXPERIMENTAL WORK

Thinking that Karyolysus might appear in apparently negative frogs, weekly examinations were made on some frogs for as many as six weeks. No frog which had an initial negative examination for Karyolysus ever showed parasites at a subsequent examination, nor did any frog which originally had Karyolysus lose the infection. Although no

actual counts were made the impression was obtained that the number of parasites remained fairly constant during the few weeks of examination. Knowing the life-cycle of Lankesterella and that it could be mechanically transmitted, it was not noted whether it appeared or disappeared in *R. clamitans,* interest being centered in Karyolysus. However, Lankesterella was never observed at any time in *R. palustris.*

An attempt was made to see if there was any periodicity in the parasites during twenty-four hours or more. For this purpose frogs were bled at hourly intervals for twenty-four hours or as long as they could stand it, but the results indicated that there was no change. The parasites were measured to see if they changed in size, but no change was found. This measuring was undertaken, because what appeared to be dividing forms were noted a number of times in red blood cells, leading to the belief that perhaps the forms were not gametocytes but schizonts. Later, mechanical transmission experiments merely confirmed the general idea that they were gametocytes, and that the divisions noted were chance happenings. Frequently red blood cells were doubly parasitized, sometimes triply, whether from these divisions or double entry of parasites or both, being uncertain.

With the hope of being able to mechanically transmit the parasite and thus follow the course of the organism within the host various experiments were tried. Frogs with Karyolysus were selected and killed. Blood was taken from the heart into a hypodermic syringe containing a small amount of citrate. This was then injected into uninfected frogs. Since the frogs which metamorphosed in the laboratory never showed any infection with Karyolysus, they were selected for inoculation as known uninfected animals. Blood from green frogs was injected intramuscularly, intraperitoneally, into the skin of the back and given by mouth to both green and bull frogs. Blood from bull frogs was likewise given to both green and bull frogs. For from six to eight days examinations were made on alternate days, then weekly for a number of weeks, but always with negative results. One exception must be made: in a few frogs for a few days a very small number of parasites could be found in the peripheral blood, but they soon disappeared. This led to the belief that they were merely some of the parasites inoculated, not the result of any multiplication within the host.

From a green frog heavily parasitized inoculations of ground-up liver, lung and spleen in citrate were made intraperitoneally with both green and bull frogs. Schizogony takes place supposedly in the endothelial cells of the blood vessels, therefore schizonts should have been present in liver, lung and spleen; but although examinations were made for a period of some weeks, no signs of infection were ever found. Material was not available to repeat this experiment.

SUMMARY

1. A survey of the haemogregarine type of parasites made at Cold Spring Harbor, showed frogs to be parasitized as follows:

(*a*) *Rana clamitans,* adult and young, was parasitized with Karyolysus and Lankesterella.

(*b*) Laboratory metamorphosed *R. clamitans* and *R. catesbiana* were parasitized with Lankesterella.

(*c*) Tadpoles of several species were parasitized with Lankesterella.

(*d*) *Rana palustris* was never parasitized with either Lankesterella or Karyolysus.

2. Frogs kept isolated over a period of weeks if not parasitized at time of capture never showed the parasite, nor did frogs parasitized at the time of capture lose their parasites.

3. Gametocytes were the forms found in the peripheral circulation.

4. Efforts to transfer mechanically Karyolysus from one frog to another were not successful.

NEW SPECIES OF COCCIDIA FROM THE SKUNK AND PRAIRIE DOG*

JUSTIN ANDREWS

In testing the cross-infectivity of coccidia from different mammalian hosts, new species of Eimeria were encountered in each of two common skunks, and in each of two prairie dogs, all of which came from an animal supply house in Ohio. The infection in the prairie dogs had disappeared when they were killed, and in the skunks the parasites had become so very scarce that no schizogonic stages could be found, though the intestines were carefully searched.

As far as can be learned from the available literature coccidia have not been described from either of these animals, although the distribution of coccidia geographically and zoologically is known to be very extensive.

Eimeria mephitidis n. sp.

Type host: the common skunk of North America.

Only the sporogonic stages have been observed. Oocysts were passed in the sporont, or unsegmented, stage (Fig. 1). Development took place readily in moist feces at room temperature, so that after a few days individuals could be found with four sporoblasts or with completely formed sporocysts and sporozoites.

The mature oocyst (Fig. 2) varied in shape from broadly oval to spherical. The average form index (average width divided by average length) was 0.9304. The wall was apparently double layered and was nearly 1μ thick. In specimens properly oriented, a circular micropyle could be seen penetrating one end of the oocyst. It was about 1.5μ in diameter. The oocyst was transparent, colorless, and contained no residuum. Fifty were measured by camera lucida at a magnification of 750 diameters. The following dimensions and biometrical data were entered as being typical.

OOCYSTS OF *Eimeria mephitidis* n. sp.

Length in Microns		17	18	19	20	21	22	23	24	25	
Breadth	16	2			2						4
in	17		1								1
Microns	18		4	1	4	2					11
	19		1	1	5	6	1	2	1		17
	20			1		1		2			4
	21				1	2	1	1	1		6
	22		1			1	1	1	2	1	7
		2	7	3	12	12	3	6	4	1	50

	Length	Breadth
Range	17–25μ	16–22μ
Mean	20.68 ± 0.19μ	19.24 ± 0.16μ
Standard deviation	1.96 ± 0.13μ	1.67 ± 0.11μ
Coefficient of variation	9.5 ± 0.6%	8.7 ± 0.6%

*From the Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland.

The sporocysts were oval with a rostrum at one end. They varied in length from 10 to 12μ and in width from 7 to 9μ. Two sporozoites and a sporocystic residuum comprised the contents of each. The two falcated sporozoites lay peripheral to the residuum, which was roughly spherical in shape and varied in size, and were twisted reciprocally so that the anterior end of one lapped over and lay upon the posterior end of the other. The length of the sporozoites, extended, was from 10 to 14μ and the width of the widest part was 4 or 5μ. The nucleus was not visible in the living specimen.

Eimeria cynomysis n. sp.

Type host: the prairie dog of North America.

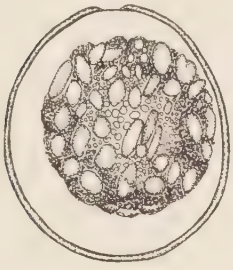
No stages of schizogony have been observed. The oocysts passed were unsegmented (Fig. 3) but developed to maturity in three or four days in moist feces left at room temperature. The ripe oocysts of *E. cynomysi* were not so nearly spherical as those of *E. mephitidis,* nor did they vary as much in their dimensions. The average form index of *E. cynomysi* was 0.8495. The wall was double and was quite thick varying from 1.5 to 2.5μ. The wall had the appearance of being fibrous and its outer surface was very irregular. It was transparent but the innermost layer was slightly tinted so as to impart a faint orange-yellow color to the oocyst. The wall was punctured at one polar extremity by a micropyle which was 5 to 6μ in diameter. There was no oocystic residuum. Twenty-five oocysts were measured by camera lucida at a magnification of 750 diameters. The following dimensions and biometrical data were entered as typical.

OOCYSTS OF *Eimeria cynomysi* n. sp.

Length in Microns		33	34	35	36	37	
	28		2				2
Breadth	29			1		1	2
in	30	1		8	7		16
Microns	31		1	1	1		3
	32					2	2
		1	3	10	8	3	25

	Length	Breadth
Range	33–37μ	28–32μ
Mean	35.36 ± 0.13μ	30.04 ± 0.12μ
Standard deviation	0.97 ± 0.09μ	0.91 ± 0.09μ
Coefficient of variation	2.7 ± 0.3%	3.0 ± 0.3%

The sporocysts were seed shaped with an inconspicuous rostrum at one end. In width, they varied from 8 to 12μ; in length, from 13 to 17μ. The sporocystic residuum was coarsely granular. The sporozoites were of an attenuated reniform shape, blunt at both ends but with one end a trifle larger than the other. At the point of greatest width, they were from 4 to 7μ wide; they varied in length from 12 to 16μ. No interior structures were seen.



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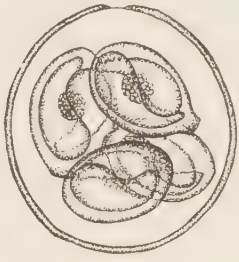

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EXPLANATION OF PLATE IX

Each drawing was made from fresh unstained material, with camera lucida at a magnification of 2,200.

Fig. 1.—Unsegmented oocyst of *Eimeria mephitidis* n. sp. from the common skunk.

Fig. 2.—Mature oocyst of *E. mephitidis*.

Fig. 3.—Unsegmented oocyst of *Eimeria cynomysi* n. sp. from the prairie dog.

Fig. 4.—Mature oocyst of *E. cynomysi*.

SOCIETY PROCEEDINGS

THIRD ANNUAL MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS, DECEMBER 27-30, 1927, NASHVILLE, TENN.

The Nashville meeting with its three day program replete with interest and its crowded sessions and vigorous discussions represents well the growth of the Society and the increasing interest in the subject. The retiring presidential address by Dr. R. P. Strong was entitled *Some Parasitic Infections Observed in Equatorial Africa During 1926 and 1927*. The background of the address consisted of a fine series of lantern slides, which gave an idea of the life of the people as well as of various parasitic conditions observed. Dr. Strong's happy combination of the popular and scientific phases of the subject was received with enthusiasm by the Society and the considerable number of visitors in attendance. He ranged from observations on malaria and sleeping sickness to nematodes of plants. His discussion of the relation of Onchocerca and other forms to tumors was especially interesting and his vivid description of the making of postmortems on elephants and hippopotomi was enthusiastically received. Since an account of the meeting has already been published in Science (February 3, 1928, page 120) only the secretary's record of the annual business meeting is given here.

The preliminary report of the treasurer for 1927 was read and approved; it was then referred to the Council for audit. It is as follows:

Deficit for 1926		$ 21.57
Dues for 1926 (paid in 1927)	$ 8.00	
Dues for 1927	409.00	
Total dues	$417.00	
Available for 1927		392.43
Expenditures to December 24, 1927		339.85
Balance available for 1927		$ 52.58

The report of the secretary showing the status of membership as of December 17, 1927, was read and approved. It is as follows:

	United States	Foreign Countries	China Branch	Total
Members in good standing (Paid for 1927)	316	61	31	408
Applications for membership accepted Dec. 17, 1927	25	2	1	28
Members delinquent for 1927	20	7	19	46
Members delinquent for 1926 and 1927	3	2	..	5
Total membership	364	72	51	487
Members lost by death, resignation, and dropping	11	5	..	16
Total elected to membership since the organization of the Society	...	..	..	503

Henry B. Ward, the Chairman of the Committee on Publication, reported that returns from the questionnaire on making the JOURNAL OF PARASITOLOGY the official organ of the Society showed 232 favorable, of whom 88 were already subscribers and 144 non-subscribers, and 49 unfavorable. This seems to show a surprisingly strong sentiment in the Society in favor of this proposition. The report was approved.

On recommendation of the Council a committee consisting of W. W. Cort, M. C. Hall and W. A. Riley was appointed to work out in conference with the editor of the JOURNAL OF PARASITOLOGY the details of a plan for making it the official organ of the American Society of Parasitologists and to report to the Society at its next annual meeting.

The report of the committee on the Teaching of Parasitology given by F. D. Barker, Chairman, was approved and the committee continued.

The nominating committee reported and on motion the persons named were unanimously elected.

President—C. A. Kofoid.

Vice President—R. W. Hegner.

Secretary-Treasurer—W. W. Cort.

Members of the Council for Four Years—W. A. Riley and W. H. Taliaferro.

Resolutions of thanks were passed commending the work of the Program Committee, the Secretary-Treasurer and the local representative of the Society in arranging for the Nashville meeting.

The meeting then adjourned.

W. W. Cort, *Secretary-Treasurer.*

CHINA BRANCH

The China Branch met at the Peking Union Medical College September 30, 1927. There was a good attendance, particularly of the younger Chinese group.

Tertian malaria in a village near Peking. C. U. Lee and H. E. Meleney.—In Ch'engfu, a village about 4 miles to the northwest of Peking, an examination of 260 children below the age of 20 showed a spleen rate of 10.4. In Dr. and Mrs. Learmonth's clinic 254 cases of tertian malaria were seen with the highest incidence in the summer and autumn months. These data show the existence of a large amount of tertian malaria in the rural districts around Peking.

Parasitic infections in the Foochow area, Fukien Province, China. E. C. Faust and C. R. Kellog.—The survey of human parasitic infections in the Foochow area, conducted during the summer of 1927, which involved an examination of approximately 500 human stools, in addition to the autopsy of reservoir and intermediate hosts of potential human parasites, showed the following conditions: (1) The small number of species and low incidence of intestinal protozoa. (2) The uniformly heavy infection with Ascaris and to a somewhat lesser degree of Trichuris. (3) The low (subclinical) incidence of hookworm infection in the area, except in the Hak Ka villages near Foochow, where a heavy infection of apparently pure *Ancylostoma duodenale* was encountered. (4) The absence of Taenia, Echinococcus, Hymenolepis and Dipylidium infection in the human population; the occasional presence of *Sparganum mansoni* in man and the possibility of human infection with the adult Diphyllobothrium due to the high infectivity of the intermediate hosts commonly consumed as food without sufficient heating. (5) The incidental infection with *Schistosoma japonicum* and *Fasciolopsis buski* in the vicinity of Foochow. (6) The complete absence of Clonorchis infection in man and in dogs and its low frequency in cats.

Report of the second case of Sparganosis from man in China. E. Campbell.—The only record of *Sparganum mansoni* from the human host in China is that of Cobbold (1883), based on the autopsy findings of Manson in Amoy the previous year. The life history investigations of the parasite by Okumura (1919), verified by Leiper (1925), shows that the first intermediate host is a Cyclops, that the usual second intermediate hosts, frogs and snakes, acquire their infection by ingesting the infected first intermediate hosts, and that the usual reservoir hosts, dogs and cats, become infected from eating raw frog and snake meat containing the Sparganum larvae. During the month of August, 1927, a patient from a neighboring village appeared at the clinic of the mission hospital at Pagoda Anchorage, Foochow, with an abscess of the thumb which looked like a felon. He gave a history of having extracted eight small ribbon-like "worms" from the granulating tissue of the infected member. Exploration of the abscess resulted in the recovery of another such "worm," which was alive and was alternately contracting and elongating. The specimen, which was believed to be a Sparganum larva, was sent to Dr. E. C. Faust, who confirmed the diagnosis. This is the second record of *Sparganum mansoni* from man in China.

C. F. Wu, *Secretary.*

Peripheral lesions in the hamsters produced by the inoculation of several strains of Leishmania. C. W. Young.—The lesions were of a deep cutaneous type never ulcerating superficially, and involving the carpel and metacarpel joints of the fore feet, the tarsal and metatarsal joints of the hind feet, and the region of the external genitalia. They contained an abundance of clasmatocytes filled with Leishmania bodies. The localization of the lesions was not due to trauma. The organisms used consisted of two strains (three cultures) of *Leishmania infantum* (vel *donovani*), one of *L. canina,* and one of *L. tarantolae,* all of which had been obtained from Dr. Nicolle of Tunis.

A new case of Isospora hominis *from China.* E. C. Faust and Lo Ke-chang.—Only one case of *Isospora hominis* has been reported from China, having been discovered in the clinic of the Church General Hospital, Wuchang (Wassell, 1923). This parasite was recovered from the stool of a patient in the out-patient department of the Peking Union Medical College Hospital on September 28, 1927. The patient gave a history of chronic diarrhea. Cysts of both the one-celled and two-celled stage were common in the feces.

HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The one hundred and fourth meeting was held on April 16, 1927. Dr. C. W. Stiles was appointed the Society's representative to the International Zoological Congress at Budapest.

Dr. E. W. Price presented the following notes:

1. The coyote (*Canis latrans texensis*), a new host for *Oncicola canis* (Kaupp) and *Oslerus osleri* (Cobbold),—Some acanthocephalid worms, collected by Miss E. B. Cram from the small intestine of a coyote, have been identified by the writer as *Oncicola canis* (Kaupp). The animal had been killed at Eagle Pass, Texas, and the viscera sent to the Zoological Division by Mr. C. P. Landon of the Bureau of Biological Survey. Parker (1909) suggested that coyotes were probably the normal hosts for this parasite, but all previously reported cases have been from the dog. One case of infestation with *Oslerus osleri* (Cobbold) was found in a coyote examined post-mortem by the writer at College Station, Texas, January 19, 1925. This parasite occurs in small tumors at the tracheal bifurcation. In this case only five small nodules were present. The only previous record of this parasite in this country was reported by Milks at Ithaca, N. Y., who found the parasite in dogs. The apparent rarity of this parasite suggests that its normal hosts are probably such wild carnivores as coyotes and wolves.

2. The occurrence of *Ostertagia bullosa* in a prong horn antelope.—Several specimens were found in the small intestine of a prong horn antelope, *Antilocapra americana,* which died in the National Zoological Park, Washington, D. C. This species was described by Ransom and Hall in 1912 from sheep from Colorado and Montana, but apparently no record of its occurrence has since been made.

3. The civet, *Bassariscus astutus flavus,* a new host for *Uncinaria lotoris.*—Several specimens of this hookworm, described by Schwartz (1925) from *Procyon lotor,* were found in the small intestine of the civet or ring-tailed cat at College Station, Texas, by the writer December 15, 1925. The finding of this species in a locality so remote from its type locality (Maryland) suggests that it is probably a common parasite of raccoons and related animals.

Dr. J. A. Scott reported as follows on the susceptibility and resistance of various animals to *Ancylostoma caninum.*

Experiments have been carried on at the Johns Hopkins School of Hygiene for the past year and a half in regard to the infection of a number of hosts with the dog hookworm, *Ancylostoma caninum.* In the dog, the normal host, the percentage of the larvae given per os which will develop varies with the age of the host from 1 to 80%, a number of older dogs being practically insusceptible. The rat has been used as an abnormal host. In it none will develop, while mature or partially mature worms, transferred to these hosts in capsules, will not establish themselves. Approximately 25,000 will kill rats with pneumonia. Cats

apparently occupy an intermediate position. They resist enormous doses, and the average percentage which develops varies from 0.1 to 0.5%. In each of these hosts a certain percentage of the worms will not develop, but do remain in the body in the condition of the infective larvae. These can be isolated by the use of the Baermann isolation apparatus. The numbers so recovered fall off rapidly from 20 or 30% of the dose after 24 hours, to from 1 to 5% after a few days. Numbers representing these lower figures can be recovered from the body up to at least 44 days in the cat, 33 days in the dog, and 21 days in the rat. Larvae which had been in each of these three hosts for about two weeks without any development were given to susceptible puppies. In every case the usual percentage developed normally, and also a small percentage remained in the body without development. Larvae from the same cats and dogs were also put in supposedly susceptible kittens. In the first trial there was no development of larvae from either cats or dogs. Undeveloped forms, however, remained in these kittens for over two weeks. When these, in turn, were given to another puppy, the usual percentage grew. Exact details, including control experiments will be published elsewhere. It seems that these facts may open new fields for the study of the nature of resistance to parasitism by these worms, and possibly indicate some of the factors necessary to development.

In discussion Dr. Hall suggested the occurrence of physiological races of *A. caninum* and pointed out the possibility that should the experiments be repeated with a "cat-strain" of the parasite different results might be obtained. In this connection Dr. Steiner noted that when a sugar beet is already heavily infested with *Caconema radicicola,* additional larvae remain in the cysts and fail to develop. This is believed to be due to the influence of a root secretion coincidental with heavy infestation by this parasite.

Dr. R. W. Hegner called to the attention of the society the fact that Steffenson, with assistance provided by the Institute of Meat Packers, is about to begin experiments to demonstrate that man can live on a meat diet. Protozoological studies will probably be made on these subjects.

Dr. Hegner also reported the results of studies made by Herbert Ratcliffe and himself on trichomonads of mammals. New species were reported from the mouth of the dog and the cat, from the intestine of the opossum and the prairie dog, and from the vagina of the monkey, *Macacus rhesus.*

Dr. Benjamin Schwartz reported *Sarcocystis* sp. from the heart of an elk, *Cervus canadensis.* The specimen was forwarded from Helena, Montana, by Dr. Hadleigh Marsh, pathologist of the Livestock Sanitary Board. The heart was riddled with cysts which are small, the largest specimens measured being 924 by 218μ and the smallest measured specimens being 236 by 67μ. The spores are kidney shaped, from 10 to 17μ long, and from 4 to 5μ in maximum width. The cytoplasm of the spore towards the pointed end is hyaline and does not stain with Giemsa stain, thus standing out in sharp contrast to the cytoplasm of the remaining portion of the spore which appears deeply stained and contains rather coarse granules. The position of the nucleus is variable. In some spores the nucleus is very close to the more rounded extremity whereas in other specimens it is located at some distance from the extremity. The nucleus appears as a clear space without a definite membrane and contains a variable number of chromatin bodies. Portions of the infected heart were fed to a lamb, a dog, rats, guinea-pigs, and mice.

Dr. G. F. White reported as follows on a method for obtaining infective nematode larvae from cultures: In searching for the adult of the nematode causing creeping eruption the useful Baermann apparatus was at first used in recovering the infective larvae from the cultures. Later, a still simpler method was devised for the purpose which, through its employment for more than a year, has proven to be altogether adequate for the problem. Darling and Fülleborn in methods they have employed made use of the fact that a number of species of nematodes tend to migrate as they approach their infective stage and the close of their free-living development. The method outlined here also makes use of this fact but in a different way.

A suitable apparatus consists of a crystallizing dish into which is poured a small amount of water, a half of a Petri dish, for holding the charcoal-feces mixture, placed within the crystallizing dish, and a watch glass as a cover. Incubation is done where a high humidity is maintained. The infective larvae migrating are trapped in the water surrounding the culture. The worm suspension is easily concentrated by pouring it into a test tube and, after sedimenting, pipetting off the supernatant water. A number of modifications of the apparatus and the methods of its use have proven very satisfactory. A fuller account will be given in another place.

The one hundred and fifth meeting was held September 17, 1927. Dr. E. B. Cram was elected president.

Dr. Hall read the following telegram:—

Will you kindly announce to the Helminthological Society that autopsies of fifty street dogs of Dallas, Texas, show many light infestations of *Ancylostoma brazilicnse*. These are less numerous than *A. caninum*. Nine cases of creeping eruption have occurred in Dallas during the summer period. (Signed) W. E. Dove.

Dr. W. W. Cort presented: "A note on lesions produced in the human skin by penetration of schistosome larvae observed at Douglas Lake, Mich."

Dr. H. W. Brown presented a note on the oxygen requirements of Ascaris eggs.

The one hundred and sixth meeting was held October 15, 1927. The following resolution was adopted:

The members of the Helminthological Society of Washington learn with profound regret of the death of Dr. Henry J. Nichols. His death is a distinct loss to science and medicine, in which fields he was a distinguished and able worker, as well as the loss to us of a highly esteemed friend. We extend our sincere sympathy to his family in their bereavement and to the Army Medical Corps in the passing of a beloved comrade.

Dr. Stiles reported on the meeting of the Zoological Congress at Budapest.

Dr. J. A. Scott reported a refinement to the method of dilution counting of hookworm larvae (to be published elsewhere).

Dr. Cobb called attention to a new species of screw nemas, Ascarophis, from the gills of the sting-ray, found by Dr. MacCallum at Woods Hole, Mass., and dwelt upon the peculiar nature of the striation of the cuticula. The transverse striae take the form of a multiple helix, so that the surface of the nema becomes that of a multiple-threaded, right-handed screw. Dr. Cobb proposed a theory of the origin of these helicoid striae from the ordinary annulation of nemas through the action of anastomosing. A description of this species of Ascarophis is being published in the Proceedings of the Washington (D. C.) Academy of Sciences.

Dr. M. C. Hall spoke on Anaplasmosis in the United States.

Attention is invited to the fact that the disease known as anaplasmosis exists in cattle in the United States and that research is urgently needed in connection with the disease by reason of its great economic importance, comparable in most respects to piroplasmosis, commonly known in this country as Texas fever. While the "marginal points" now regarded by most authorities as Protozoa belonging to the genus Anaplasma were found by Smith and Kilborne in their classical investigations on Texas fever carried out in the Bureau of Animal Industry in 1889, and consequently have been known to be present in cattle in this country for almost half a century, little attention has been paid to anaplasmosis in this country. The disease has usually been present, apparently, as a complication of piroplasmosis, and as both diseases were carried, so far as known, by the cattle fever tick, *Boophilus annulatus*, and as measures for the control of piroplasmosis have been along the line of a well organized campaign for the eradication of this tick, it has been assumed that tick eradication for the control of piroplasmosis would also control anaplasmosis. The assumption that tick eradication was driving back and stamping out both diseases has seemed to be a fair assumption for twenty years. Systematic tick eradication was begun in 1906 and it was not until 1926 that the first evidence appeared in print indicating that a residuum of anaplasmosis was being left behind the advancing wave of tick

eradication. In 1926 Dr. Paul B. Darlington, a veterinarian of Chanute, Kansas, reported anaplasmosis in cattle in Kansas, not only far in the rear of the tick quarantine area, but even north of the old 1906 quarantine line. Following this it was reported by Dr. Douglas and Dr. Flower from the vicinity of New Orleans, Louisiana, and by Dr. Boynton and others of the University of California and Dr. Rudolph Snyder of the Bureau of Animal Industry in various parts of California. Recently it has been reported from Florida and Oklahoma in areas free from the cattle fever tick. In all probability the disease will be found to be widely distributed.

The occurrence of anaplasmosis in areas free from fever ticks indicates that some vector other than the fever tick must be sought for as one of the carriers of the etiological agent of anaplasmosis. The reports to date show that the diseased cattle are tick-free, apparently uninfested by ticks of any sort. Heretofore the only carriers known for Anaplasma have been ticks, so far as the matter has been investigated in South Africa, South America or elsewhere. The carriers reported include *Ixodes ricinus, Boophilus annulatus, B. annulatus decoloratus, B. a. australis, B. a. microplus,* and *Rhipicephalus simus.* There is a possibility that in this country anaplasmosis is being transmitted by some vector other than ticks, and it is conceivable that a biting fly might serve as a mechanical carrier for a limited portion of the year, the disease flaring up seasonally by transmission from chronic carriers to new and susceptible hosts. This is an important aspect of the matter which needs investigation. Anaplasmosis has a high mortality, and up to the present time there is no satisfactory treatment. The trypanblue treatment which is of value in piroplasmosis is not of value in anaplasmosis. Here is a field for research in which the results will be especially valuable if it is found that anaplasmosis in this country can be conveyed by such vectors as biting flies, since fly control may prove to be a very difficult thing or even impracticable in the present state of knowledge. Since investigations have shown that a bilirubinemia associated with jaundice may cause a dangerous exhaustion of the ionized blood calcium, it is possible that intravenous calcium may prove beneficial in this anaplasmosis.

The intent of this note is to bring the subject of anaplasmosis in the United States to the attention of American investigators as a problem of great scientific interest and economic importance. It is a problem which offers many phases of investigation to the general parasitologist, the protozoologist, the entomologist and the veterinarian.

The one hundred and seventh meeting was held at the School of Hygiene and Public Health, Johns Hopkins University, November 19, 1927.

Dr. E. W. Price presented the following list of helminth parasites occurring in Texas, based upon specimens from animals examined postmortem at the School of Veterinary Medicine, A. and M. College of Texas during the years 1919 to 1926. These animals were from the vicinity of the college and in view of the fact that this section is not a livestock section, the number of species found is rather remarkable. The species listed from man are based upon a small collection received from local hospitals. In one lot of material from the Bryan Hospital two specimens of *Taenia solium* were found. These are of especial interest as this parasite is rarely found even in localities where the larval form, *Cysticercus cellulosae,* is common.

Horse and mule.—*Anoplocephala magna, A. mamillana; Parascaris equorum, Oxyuris equi, Probstmayria vivipara, Strongylus equinus, S. edentatus, S. vulgaris, Gyalocephalus equi, Triodontophorus tenuicollis, Cylicostomum* spp., *Dictyocaulus arnfieldi, Trichostrongylus axei, Habronema megastoma, H. microstoma, H. muscae, Onchocerca cervicalis, Setaria equina.*

Cattle.—*Paramphistomum cervi, Fasciola hepatica, F. magna; Moniezia planissima, Cysticercus tenuicollis, Cysticercus bovis; Trichuris ovis, Proteracrum radiatum, Bunostomum phlebotomum, Haemonchus contortus, Trichostrongylus axei (= T. extenuatus), Cooperia punctata, Ostertagia ostertagi, Dictyocaulus viviparus, Gongylonema scutatum, Setaria labiato-papillosa.*

Sheep and goats.—*Fasciola hepatica; Moniezia expansa, Thysanosoma actinioides, Cysticercus tenuicollis; Trichuris ovis, Proteracrum columbianum, Bunostomum trigonocephalum, Trichostrongylus axei, T. colubriformis, Haemonchus contortus, Nematodirus spathiger, Cooperia curticei, C. oncophora, C. punctata, Ostertagia circumcincta, Dictyocaulus filaria, Gongylonema scutatum, G. verrucosum.*

Swine.—*Cysticercus cellulosae, Echinococcus granulosus; Trichuris trichiura (= T. suis), Ascaris lumbricoides, Oesophagostomum dentatum, Globocephalus urosubulatus, Hyostrongylus rubidus, Metastrongylus elongatus, M. salmi, Choerostrongylus pudendotectus, Stephanurus dentatus, Gongylonema ransomi, Arduenna strongylina, Physocephalus sexalatus; Macracanthorhynchus hirudinaceus.*

Dog.—*Alaria* sp.; *Dipylidium caninum, D. sexcoronatum, Taenia hydatigena, T. pisiformis, Multiceps serialis; Trichuris vulpis, Toxocara canis, Toxascaris limbata, Ancylostoma caninum, A. braziliense, Oslerus osleri, Dirofilaria immitis, Spirocerca sanguinolenta; Oncicola canis.*

Cat.—*Taenia taeniaeformis, Dipylidium caninum, D. sexcoronatum; Toxocara mystax, Ancylostoma caninum.*

Man.—*Taenia sagiata, T. solium, Hymenolepis nana; Ascaris lumbricoides, Enterobius vermicularis.*

Rabbit.—*Hasstilesia tricolor; Cittotaenia variabilis, Davainea salmoni, Cysticercus pisiformis, Multiceps serialis; Obeliscoides cuniculi, Trichostrongylus calcaratus, Nematodirus leporis.*

Chicken.—*Capillaria* sp., *Ascaridia lineata, Heterakis vesicularis, Cheilospirura hamulosa.*

Ostrich.—*Libyostrongylus douglassii, Codiostomum struthionis.*

Benj. P. Young spoke on infestation and the prepatent period of *Eimeria avium* in chicks. At the present time two results seem well established from study of host-parasite relations between the coccidial protozoon, *Eimeria avium,* and the chick. The prepatent period, or the time elapsing from the entrance of oocysts into a new host until the appearance of others in the feces, has been determined for young barred-rock chicks to be approximately four days for those inoculated with segmented oocysts. Infections may occur when chicks are inoculated with unsegmented oocysts a few hours after these are passed in the feces of other birds. The prepatent period for birds inoculated with recently voided unsegmented oocysts is slightly over five days. Since sporulation in the laboratory requires scarcely more than twenty-four hours the greater length of the prepatent period when unsegmented oocysts are inoculated into chicks is probably due to the time necessary for the development of the oocysts to the sporozoite stage.

Dr. N. A. Cobb described and exhibited drawings of a new species of nema from earthworms. He also called attention to work recently done at Woods Hole, Massachusetts, making it the basis of an appeal to members to point out to friends and colleagues how promising a field the mermithid parasites of insects present from many scientific standpoints.

Charles Rees presented a note on the effects of rice starch on the growth and pathogenicity of *Endamoeba histolytica.* A modification of Boeck and Drbohlav's (1925) medium for the cultivation of *E. histolytica* is described by Dobell and Laidlaw (1926). It consists in the addition of a few particles of sterile rice starch to each culture tube. This modification of the medium has been used here for the past two months. The statements of Dobell and Laidlaw that the growth of the endamoebae is accelerated has been amply confirmed. It has been found also that the starch fed organisms possess greater viability than those not so treated. An accidental increase in the temperature in the incubator from 36°C. to a point a trifle over 37.5°C. recently occurred. Despite much care the organisms died out in those cultures reared without starch whereas those receiving starch survived. Dobell and Laidlaw also claim that the organisms grown in the modified culture tubes lost their pathogenicity for kittens. Eight kittens receiving rectal injections of fluid rich in endamoebae failed to become infected or manifest symptoms. The

method of rectal injection has not been tested here but the laparotomy method, as described by Sellards and Theiler (1925), has been employed. It has been found that the feeding of starch does not destroy the pathogenicity of the parasite. Of six kittens receiving injections of the organisms directly into the ligated colon and killed at intervals of 48 to 140 hours, five became infected and in the colons of three of these there were lesions. In sections of these lesions it was found that *E. histolytica* had penetrated as far as the muscularis mucosae. Only two of 9 kittens killed in less than 48 hours became infected and in neither of these had lesions developed. The endamoeba used here had been subcultured in starch for thirty days at the time of the last experiment, whereas Dobell and Laidlaw's organisms had been subcultured over 38 days. But it is evident from these experiments that starch ingestion is not a factor that destroys pathogenicity in *E. histolytica.*

Oliver R. McCoy presented a note on seasonal fluctuation in the infestation of *Planorbis trivolvis* with larval trematodes. (To be published in this JOURNAL.)

Justin Andrews spoke on two new species of coccidia from skunks and prairie dogs (this JOURNAL, page 193).

Herbert Ratcliffe spoke on the relation of intestinal P_H to the intensity of trichomonad infections in albino rats. The number of *Trichomonas muris* present was studied over a period of 16 days in five rats, all females six months of age that had been on a starch (McCollum) diet for three months. Every four days 0.2 cc. of cecal material was removed and diluted (1-10) in normal saline. The P_H was determined and the flagellates counted with a blood counting chamber. In all cases there was a close correlation between the P_H and the number of flagellates; 6.6 appeared to be the optimum and counts varying between 5,000 and 40,000 flagellates per cu. ml. were found at this point. In cases falling below or above 6.6 there was a marked decrease in the number of trichomonads. At 6.2 and again at 7.4 the smallest counts, falling below 500 per cu. ml., were found. It is also of interest to note that there was an individual variation in intestinal P_H in some of the animals while in others it remained constant throughout the experiment. In all cases of variation there was a close correlation between the changes in P_H and the number of flagellates present.

Eugene Schumaker gave a report on Iodamoeba in a chimpanzee. Cysts of an Iodamoeba having an average diameter of 7.4μ and also a trophozoite were discovered in smears made from the intestinal contents of a chimpanzee which died in Druid Hill Park Zoological Garden, Baltimore, Maryland, during the summer of 1927. This appears to be the first report of a member of this genus in the chimpanzee, although iodamoebae have been described from other primates.

Elizabeth P. Sanders reported that infections with three different species of Protozoa indicate that these organisms cause characteristic changes in the blood of kittens. Kittens inoculated with *Trypanosoma equiperdum* developed anemia, a marked general leucopenia and a terminal hypoglycemia. Kittens inoculated with *Endamoeba histolytica* developed, during the course of a fatal acute amoebiasis, a slight anemia, and a polymorphonuclear leucocytosis coupled with a rise in the number of large mononuclear cells. In the naturally acquired infections of coccidiosis found in all kittens used, there seemed to be a lymphocytic leucocytosis during the prepatent period followed by a slight lymphocytosis during the course of the infection.

Dr. Robert W. Hegner spoke on the ingestion of red-blood corpuscles by trichomonads. Trichomonads from the intestine of man, mouth of man, intestine of monkey, vagina of monkey, cecum of rat, cecum of chicken and rectum of frog were grown in serum-saline-citrate medium to which was added fresh blood from man, rabbit, guinea-pig, dog, cat, rat and mouse. All types of trichomonads ingested all types of red-blood cells indicating that the red cells are accepted as adventitious food material, and that the ingestion of red-blood cells is not evidence of pathogenicity.

Dr. E. B. Cram reported that there have been sent to the Zoological Division recently, for identification, two lots of specimens which had been regarded as parasites by their collectors. The one lot consisting of small, dark, wormlike

objects, collected from the feces of a 14-month child, proved to be the fibre cells of the banana, the arrangement of the cells resembling a tapeworm strobila. With the increased use of the banana in the diet of infants, an acquaintance with the harmless nature of these indigestible fibre remnants will prevent cause for alarm. The second case consisted of numerous small, thin-walled bladderlike objects passed by a dog after the administration of a vermifuge. These spurious parasites were identified as the pulp vesicles of citrus fruit. Previous reports of these vesicles being considered of parasitic nature have all dealt with material derived from man, as far as is known to the writer, this being the first case in veterinary medicine.

Dr. Benjamin Schwartz presented the following notes.—1. A nodular disease of the intestine of the carabao in the Philippine Islands due to an undescribed species of the nematode genus Cooperia. 2. *Cysticercus cellulosae* was fed to three dogs which were killed 4 days, 10 days and 60 days, respectively, after feeding. In four days the worms showed some development and in ten days they showed considerable growth. No tapeworms were found in the dog killed 60 days after feeding.

Dr. M. C. Hall presented a note on condemnation of parts of swine in meat inspection for parasitism. Dr. Nighbert and Mr. Connelly were instructed to keep a record at Moultrie, Georgia, on parts of swine carcasses condemned for parasitism. Their report shows: Over 5,000 swine slaughtered; over 19,000 pounds of livers, kidneys, kidney fat, sweetbreads and casings condemned; net loss over $1,500 for one week, or a loss of 28⅓ cents per head.

J. R. Christie, *Secretary.*

BOOK REVIEWS

An Introduction to Medical Protozoology. By Robert Knowles, 887 pp., 174 figs., 15 colored plates. Thacker, Spink & Co., Calcutta.

This textbook was written primarily for use in the author's classes. Seemingly there is no end of such texts but this one should have a much wider circulation than the usual work by reason of its worth. The author has for a number of years been studying the principal medical protozoa about which he is writing. He acknowledges extensive copying ("loot") from other sources, chief of which is Wenyon (1926), but from his comment the extent of this copying might be somewhat overestimated as shown by the fact that out of one hundred bibliographic references taken at random, sixteen could not be found in Wenyon's work. Of these sixteen most were either to be found in Indian journals or were very recent. The author's objective of providing a textbook not "beyond the purse of the Indian student," and not "to voluminous for him to study" has been well achieved, although his views as to what is "too voluminous" might be open to some question. The subjects are so completely covered in the text that, as one may well surmise, only those of his students take lecture notes who do not like to read.

The work is arranged in nineteen lectures on different Protozoa, seven chapters on laboratory methods, ninety-one pages of references and an index. While it would at the present time be unreasonable to expect and undesirable to have complete agreement on all points in medical protozoology, some American workers will find ample opportunity for disagreement with details and will also note evidences of certain likes and dislikes on the part of the author. He lists as a major reason against any attempt to change the generic name *Leishmania* the chance that thus Sir William Leishman might soon be forgotten. The mistakes of Schaudinn are minutely considered—but how many more textbooks must be written before the major part of his efforts can be peacefully ignored! The author is more specific than Wenyon in his claim that the discovery of the punctate markings to be found in red cells parasitized by *Plasmodium falciparum* should be credited to Stephens and Christophers rather than to Maurer who has usually been regarded as the discoverer. The general policy of the author in regard to species leans toward "lumping" rather than "splitting" but there does not seem to be any fixed rule for the recognition or rejection of a species. The author may, as noted above, ask his readers to accept as falciparum a strain of malarial organisms which did not form crescents; and then in another place he uses *bütschlii* as the specific name of the *Iodamoeba* of man. In order to do so he must fail to recognize that two independent workers reexamined the original material to which the name *williamsi* was given and found that it contained the Iodamoeba of man and *Endamoeba coli*.

Looking at the work as a whole, there is evidence that the book was hastily gotten together, which the author admits and regrets. The surprising thing is not that there are mistakes but that there are so few considering that the whole book including illustrations was assembled in nine months. Under such conditions it was obviously impossible to abstract and critically examine all the original publications. The best that he could hope for, was to take the findings of some of the workers who have recently reviewed the various topics. However, the selection of these recent articles was not in all cases equally fortunate.

As the title states, the book is primarily a medical protozoology since the major emphasis is laid on those forms which are important etiologically or which may be encountered in man. As such it is to be recommended to the medical profession as being the most up-to-date and usable book on the market at present. It is very well illustrated—in fact, illustrations seem to have been so plentiful that one full page figure (Paramecium) was included both near the beginning and again

near the end of the book. The descriptions, illustrations and laboratory directions make it possible for the average trained medical man with proper equipment to identify the protozoa and spirochaetes he may encounter. Treatment is, as it should be, largely left to treatises on medicine and pharmacology. The book is specifically intended for the tropics and malaria, leishmaniosis, kala-azar and trypanosomiasis receive most thorough treatment; but this does not rob it of value for the doctor in the temperate zones for he needs to be prepared for such diseases.

Collected Addresses and Laboratory Studies, Vol. III, 1926-27. Compiled by R. T. Leiper (London School of Hygiene and Tropical Medicine).

This volume includes contributions from the school during the academic year ending July, 1927. It embraces four fine addresses by the Director, Dr. Andrew Balfour, and fifty-four laboratory studies by forty odd authors. All are reprints of papers originally published elsewhere and are reproduced from a considerable number of different scientific journals. The series illustrates well the amount and variety of work undertaken by the school. It covers contributions to the knowledge of sprue, Addisonian anemia and other diseases of man, together with work on parasites from a wide variety of hosts. Helminthology is most abundantly represented. There are papers on the morphology, distribution, taxonomy, experimental treatment and life history of various types of worms and protozoa. Entomology, also, is included and contributions on human and comparative pathology form an important part of the volume.

Tercera Reunion de la Sociedad Argentina de Patologia Regional Del Norte, Tucuman Julio 7, 8 y 10 de 1927. 779 pp.; many text figs. and full page plates. Buenos Aires.

An imposing volume of nearly 800 pages presents the papers of this meeting which divided its activities among 12 sections. Substantially no space is wasted on the dreary detail of convention procedure, committee organization or routine reports, but the entire volume is devoted to scientific papers. While all the work is interesting in general and every part has its relations to the field of medical zoology, yet the parasitologist will find particular profit in the sections on uncinariosis, dysenteries and diarrhoeas with reports on Balantidium and Isospora, leishmaniosis, malaria, medical entomology, and human and comparative parasitology. In the last noted section are papers on microfilariae, Rhinosporidium, hydatid cysts, Piroplasma, Sparganum, Haemoproteus, trypanosomes, intestinal amoebae, and extra-intestinal taenias, as well as on other important topics. While some of the most distinguished of European parasitologists are recorded as contributors, the major part of the volume was written by South American workers. The book is thus a most significant demonstration of the progress of scientific, especially biological research on the South American continent.

Mosquito Surveys. A Handbook for Anti-Malarial and Anti-Mosquito Field Workers. By Malcolm E. MacGregor. 282 pp., 3 maps and 59 other illustrations. William Wood & Company, New York.

In his foreword Sir Ronald Ross characterizes this as a "complete and important book" and emphasizes rightly the significance of malaria which alone not only during a few years but for all times past and present, has been responsible for "a mortality equal to that of the Great War at which we stand aghast." The author who has had wide experience at home and abroad with malaria and anti-malaria measures has been investigating disease-carrying insects at the Field Laboratory of the Wellcome Bureau of Scientific Research at Wisby. Accordingly he comes to the subject with a fund of experience which is rarely equalled.

The book takes up first the morphology of the mosquito in general, and its life history. Part II is devoted to the scientific study of the characters and bionomics

of the mosquitoes of Mauritius and Rodriguez. Here the notes on these species are very full and of great value to the field worker. In Part III the author considers laboratory and field technique. The book is well written; despite its brevity which has brought an immense amount of detail within the scope of 282 pages, the style is attractive and clear. One may congratulate the printers on the appearance of the work which in type, illustrations and general make up adds much to the appeal of the text.

RECENT ADVANCES IN TROPICAL MEDICINE. By SIR LEONARD ROGERS. 398 pp., 12 illustrations. P. Blakiston's Son & Co.

In his preface the author begins by admitting that "Tropical Medicine is not now making such giant strides as it did two or three decades ago, yet much detailed progress is reported yearly from widely scattered parts of the world." The work covers a wide range of subjects, most of which are of interest to the parasitologist and at least three quarters of the book cover the relations of animals to disease albeit that, as the author states, especial prominence has been given to treatment and to other points of most practical import to the isolated medical man in the tropics. While perhaps the pages of the best abstract journals would yield all the information contained in this volume, yet students of the subject in the laboratory as well as in the field are indebted to Dr. Rogers for this well digested, and attractively presented summary of the recent work.

The Liverpool School of Tropical Medicine has issued as Memoir 3 an *Illustrated Guide to the Anophelines of Tropical and South Africa* which deserves notice. It is concise, well written and abundantly illustrated. Those who are working in that territory or need to handle material coming from it will find this work indispensable.

Professor O. Fuhrmann has published a memoir on the *Cestodes,* as Part 17 of the *Catalogue of the Invertebrates of Switzerland.* The work is admirable in style and method. It contains numerous indexes, an extensive bibliography and carefully wrought out descriptions which will be useful to workers far beyond the limits of the beautiful mountain republic.

DIE ERREGER DES FLECK- UND FELSENFIEBERS. BIOLOGISCHE UND PATHOGENETISCHE STUDIEN. MAX H. KUCZYNUSKI. Verlag von Julius Springer, Berlin, 1927, 256 pages.

This monograph records the author's own observations on the study of typhus and Rocky Mountain spotted fevers in collaboration with Wanda Blühbaum and Elizabeth Brandt. Since the volume is more a report of experimental work than a text or reference book, it should be abstracted rather than reviewed. Such an attempt would require too much space. The author has presented a useful summary at the end of the book wherein he has given the salient facts revealed by his experiments. He has apparently made no serious effort to review the literature for only a few references appear at the bottom of the pages. The volume is well printed, the illustrations are clear and its contents should be known to all investigators in this field.

OBITUARY

With deep regret the JOURNAL has to record the death on November 15, 1927, of the distinguished Italian parasitologist, Francisco Saverio Monticelli. At the time of his death Professor Monticelli was Director of the Zoological Institute of the Royal University of Naples. In earlier years he was an earnest student of parasitology and his contributions to the subject form an important part of the foundation of knowledge in this field.